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Laboratory Diagnosis of Invasive Candidiasis

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SCOPE OF THE PROBLEM

Definitions of Invasive *Candidiasis*

Diagnosis of infections due to *Candida* species presents unique problems. These organisms commonly colonize human skin and mucous membranes so that merely isolating them in culture specimens from these sites is not proof of invasion (82). In acute cutaneous candidiasis, organisms penetrate only the outer layers of the squamous stratum corneum of the skin. A pustular dermatitis results that actually more resembles a hypersensitivity reaction than an infection (68). Because of this, the term invasive candidiasis is commonly used to denote penetration of *Candida* spp. into tissues below epithelial surfaces or infection of viscera. This term distinguishes these more serious infections from heavy colonization with or without superficial penetration of an epithelial barrier. Most researchers studying candidiasis have developed case definitions of invasive candidiasis which are used to assess the utility of a particular diagnostic or therapeutic approach to the infection. These case definitions may influence the reported sensitivity and specificity of a diagnostic test. Most would agree that invasive candidiasis is present when *Candida* organisms are recovered from multiple blood cultures drawn over a period of days, when the organism is cultured from or demonstrated histologically in tissue biopsies, or when it is recovered from a normally sterile fluid such as cerebrospinal or joint fluid. However, clinicians frequently encounter situations that are much less clear than these, when the diagnosis may be considered likely enough for therapeutic intervention even though it is not proven, for example, a patient with only a single positive blood culture for a *Candida* sp. and retinal lesions typical of those seen with bloodborne dissemination to the eye or a febrile patient who has failed to respond to antibacterial drugs who has the organism growing from multiple sites including abdominal drains placed during surgery. Clinical correlations between laboratory findings and the patient's condition are essential to diagnose invasive candidiasis accurately. Physicians may need to rely on cumulative information from multiple specimens to have enough confidence to begin antifungal therapy for invasive candidiasis.

Taxonomy and Growth Characteristics

The genus *Candida* is one of the most important genera of medically important yeasts. Although any *Candida* species can produce human disease, *Candida albicans* is the most important pathogen, followed by *C. tropicalis* (76, 82, 94). Except when otherwise indicated, the term candidiasis will refer to infections produced by members of the genus *Candida*. There has been a debate among mycologists with respect to whether or not the genera *Candida* and *Torulopsis* should be fused into a single genus. Recently, it has been recommended that the medically important yeast *Torulopsis glabrata* be renamed *Candida glabrata*. The arguments for and against this are summarized well in the recently published monograph by Odds (82). *The Manual of Clinical Microbiology* (fourth edition) uses the name *Torulopsis glabrata* (22), and it is my impression that most clinical laboratories prefer to use this name in reporting culture results. Nevertheless, *Candida glabrata* will be used in this paper to simplify tabulation of information about this and the other yeast species being discussed.

Under standard conditions with optimal nutrients, these yeasts grow in log phase as budding cells (blastoconidia), which are oval to oblong and measure approximately 2 to 5 by 3 to 7 μm. The cells occur singly or in clusters or chains. On a solid medium such as Sabouraud dextrose agar, they produce moist creamy colonies that lack the fluffy velvety texture seen with colonies of filamentous fungi. However, except for *C. glabrata*, all produce pseudomycelia under

appropriate conditions. Pseudomycelia are formed when blastoconidia produce elongated buds that do not split off but remain attached to neighboring cells. Diminished oxygen tension, 5 to 10% carbon dioxide, 37°C incubation temperature, relative glucose starvation, and a rich protein milieu favor pseudohypha production, explaining the fuzzy appearance beneath colonies of *Candida* spp. growing on the surface of blood agar. These conditions also favor germ tube production by *C. albicans* and *C. stellatoidea*. Germ tubes are cylindrical filamentous structures that sprout from the side of a blastospore, grow at their tips, and show no indentation at their site of attachment to the blastospore. On suitable laboratory media, *C. albicans* germ tube growth can lead to production of a mycelium bearing chlamydospores. The surface of host mucous membranes and tissues provides conditions favoring production of pseudomycelia and germ tubes so that these filamentous growth forms are commonly encountered in Gram-stained smears from patient specimens. The organisms are variable in their staining reaction but are predominantly gram positive. In host tissues, filamentous forms undoubtedly enhance tissue penetration; however, the yeast's morphology in Gram-stained smears cannot be used to determine whether or not the specimen is from a site of tissue invasion. The filamentous growth form simply reflects the milieu in which the organism is growing.

Spectrum of *Candida* Infections

By the first two decades of the 20th century, the broad spectrum of *Candida* infections was being appreciated. Trivial self-limited or readily treatable mucocutaneous infections are common throughout the world (51). Rare genetic defects in immune response are manifested by chronic mucocutaneous candidiasis. In the past three decades, with the use of potent antibacterial, immunosuppressive, and cytotoxic drugs, lethal invasive candidiasis in compromised hosts has been described with increasing frequency (78, 93). These infections have sparked great interest in all aspects of *Candida* infections, including their diagnosis.

The many types of *Candida* infections and the diverse clinical settings in which they are found would seem to make it impossible to classify them in any meaningful way. However, certain useful generalities apply. *C. albicans* is part of the flora of the birth canal, and it colonizes the intertriginous skin and the gastrointestinal tract soon after birth. The largest pool of this organism within the host is in the gastrointestinal tract (82). Because it is an aerobe, it most likely resides in the gut lumen close to mucous membrane surfaces where it scavenges oxygen from the mucosa. The organism is clearly poised to invade the host if defects in cutaneous or mucosal barriers occur. Defects in T-lymphocyte immunity (congenital or acquired defects, as in acquired immunodeficiency syndrome) or qualitative defects in neutrophil function (myeloperoxidase deficiency) are associated with chronic mucocutaneous infections. Patients with these defects can adequately halt proliferation and invasion of the organism but cannot eliminate it from the skin or mucous membranes. Thus, invasion deeper than the skin or mucous membranes and bloodborne dissemination occur rarely in these patients.

Deeply invasive infections with dissemination occur readily when an epithelial barrier is completely breached by the fungus or when quantitative defects in neutrophil function are present. The latter can be defined as a peripheral blood neutrophil count of $<500/mm^3$. Infections produced after a gross breakdown of a normal epithelial barrier are encountered most frequently in surgical and intensive care unit patients. For example, the epithelial barrier may be breached when the organism tracks along the external surface of an intravascular catheter or is introduced directly through it into the bloodstream (63). Leakage of a surgical enteral anastomosis with soilage of the peritoneal cavity is a more obvious example of breakdown of the normal mucosal barrier (106). Dissemination can occur when vessels in the visceral or parietal peritoneum are invaded. In these instances, blood containing the organisms must traverse capillary beds of the lung and an extremity before it can be sampled by a peripheral venous blood culture. Because of their large size, many *Candida* organisms are trapped in small capillaries. Therefore, blood culturing may be less effective in detecting *Candida* spp. as compared with bacteria in the bloodstream of infected patients.

Patients on medical wards may develop candidiasis from infected intravascular catheter sites; however, their infections more commonly arise by mechanisms that make diagnosis of disseminated candidiasis even more difficult. Many of these patients have quantitative neutrophil defects. Patients at highest risk for invasive candidiasis are those with acute leukemia who are undergoing chemotherapy and bone marrow transplant recipients who have not yet shown engraftment of transplanted marrow. The cytotoxic drugs used to treat their malignancies retard repair of mucous membrane defects and the antibacterial therapy they receive alter the normal flora, thereby enhancing the growth of *Candida* spp. Infections of these patients most often begin with invasion deeply into the small or large bowel wall (33, 80). When fungi invade capillaries and veins of the gut wall, they can enter the portal venous blood and be conveyed to the liver. The liver acts as a strong barrier to dissemination because its macrophages are very efficient in removing *Candida* spp. (7, 50, 99, 114). Therefore, few organisms may reach the heart and systemic circulation, and detection of *Candida* spp. in peripheral blood cultures of these patients is difficult. Although few yeast cells may be circulating in the bloodstream, paradoxically, those *Candida* cells that reach distant sites proliferate readily because of the profound neutropenia. Thus, severe disseminated disease can occur even though blood cultures are negative.

USE OF CULTURES IN DIAGNOSIS

Need for Identifying *Candida* Isolates to Species

Most large hospitals and clinics in the United States now treat significant numbers of immunosuppressed patients, the population in which invasive candidiasis is frequently encountered. Many laboratories have not routinely identified *Candida* isolates to species, but the growing importance of severe candidiasis has made it necessary for them to do so. When coupled with information about the patient's risk factors, species identification can provide a diagnostic clue to the source of infection. For example, *C. albicans*, *C. tropicalis*, and *C. krusei* are most capable of colonizing and invading the gastrointestinal tract and producing bloodborne dissemination (74, 76, 121). *C. parapsilosis* is adept at colonizing skin, intravascular devices, and equipment used to administer parenteral fluids (108, 109). Outbreaks of *C. parapsilosis* fungemia associated with parenteral nutrition have been described (109). *C. lusitaniae* is now emerging as an opportunist and can be relatively resistant to amphotericin B (45). This organism can be misidentified as *C. tropicalis*, *C. parapsilosis*, and even *Saccharomyces cerevisiae* unless careful species identification is done.

Detailed protocols for identification have been described (22), and several commercially available products make species identification convenient. The germ tube test remains a quick and easy test to distinguish *C. albicans* from other species. *C. stellatoidea* can also give a positive germ tube test. Recent studies of the genomes and virulence of *C. albicans* and *C. stellatoidea* indicate that many strains of the species are very closely related in any event, and many taxonomists would consider germ-tube-positive *C. stellatoidea* to be simply a sucrase (α-glucosidase)-negative *C. albicans* (60). *C. albicans* can be recognized by its ability to produce a mycelium and chlamydospores on commercially available RIOT (rice-oxgall-Tween 80) medium. Multicompartmented plates (e.g., Uni-YeastTek, Flow Laboratories Inc., McLean, Va.; Yeast Ident or API 20C, Analytab Products, Plainview, N.Y.) contain a panel of indicator media that can be used to assess sugar assimilation reactions as well as yeast growth on RIOT medium. Automated instruments such as the Vitek (Vitek Systems, Inc., Hazelwood, Mo.) and Quantum II (Abbott Laboratories, Irving, Tex.) feature yeast biochemical kits for species identification.

Cultures of Sputa, Urine, and Body Fluids

Candida species grow well on blood agar; therefore, they are most often isolated from specimens submitted for routine bacterial culture rather than for fungal culture. It is important for the laboratory to report growth of *Candida* spp. from such specimens even when it is part of a mixed flora. Isolation per se may be only a sign of colonization; however, growth of the organism from multiple sites can be important for the clinician. For intensive care or burn patients, the risk of disseminated candidiasis goes up as the number of sites positive for *Candida* spp. increases (16, 107). Thus, reports of growth from multiple sites may prompt topical or even systemic therapy for some patients.

Pulmonary candidiasis occurs rarely in immunosuppressed patients and almost never in normal hosts. It was encountered in <3% of autopsies reported from several major cancer treatment centers in the late 1970s (97). Authors of the three most complete reviews of clinical experience with pulmonary candidiasis (70, 77, 97) have all emphasized that isolation of *Candida* spp. from sputa cannot be used to diagnose pulmonary candidiasis. Because endotracheal tubes will be colonized with oropharyngeal flora, they considered that isolation of *Candida* spp. from endotracheal secretions is also not a useful diagnostic criterion.

The clinical interpretation of *Candida* spp. isolated from an abdominal drainage site is difficult. Determining whether the organism either is merely colonizing the drain tubing or sinus tract or is emanating from an infected site within the abdomen may be impossible. However, there is evidence that an isolate obtained from an intraoperative specimen must be considered as producing an infection in many instances. Solomkin et al. (106) described 56 cases in which peritonitis was discovered at the time of surgery and a *Candida* species was isolated from intraoperative cultures. Thirty patients were undergoing surgery for a perforated viscus or intestinal necrosis. In the remainder, peritonitis developed after elective intraabdominal surgery and exploratory surgery was then required. *C. albicans* was recovered in 46 cases, and *C. tropicalis*, *C. krusei*, *C. parapsilosis*, or *C. glabrata* was found in 16. In 46 cases, a *Candida* sp. was cultured as part of a polymicrobial flora. Overall mortality was 70%, with uncontrolled sepsis the main cause of death. Postmortem findings in 27 patients showed 9 cases of clinically unsuspected disseminated candidiasis, and persistent intraperitoneal *Candida* infection was present in 8 more patients. None of the patients treated with amphotericin B prior to death had residual foci of *Candida* infection. In 40 patients, a *Candida* sp. was cultured from the peritoneum before the onset of multiple organ failure syndromes, and survival was related to provision of adequate antifungal therapy before blood cultures became positive. Of 6 patients treated prior to the development of positive blood cultures, 5 survived, whereas only 9 of 27 untreated patients survived. Based on this study and others (16, 107), many clinicians would give antifungal therapy to a patient with an intraoperative culture growing a *Candida* sp. Thus, it is imperative that the laboratory report isolations of *Candida* spp. from intraoperative samples even when multiple bacterial species are isolated from the same specimen.

Septic arthritis due to *C. albicans* is well described (82). Cases of prosthetic joint infections due to *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. glabrata* have been reported (61). Thus, *Candida* species growing from joint fluid aspirates should not be dismissed as contaminants.

Candida species are commonly isolated from the urine. Few data are available to judge how to interpret the clinical significance of quantitative cultures. In one study, in nine patients in whom renal candidiasis was documented by autopsy or biopsy, midstream or single straight catheterized urine specimens grew 10,000 to 40,000 *C. albicans* per ml with a mean of 22,000/ml (128). In ten patients with no renal pathology, specimens grew 2,000 to 15,000 *C. albicans* per ml. However, in patients with long-term indwelling Foley catheters, up to 100,000 CFU/ml were seen in patients with or without documented renal candidiasis (128). In intensive care unit patients with a Foley catheter in place, the presence of >100,000 *Candida* spp. per ml along with numerous leukocytes or erythrocytes may be an indication for continuous or intermittent bladder irrigation with amphotericin B. In a prospective study of urine cultures from hospitalized patients, *Candida* spp. were noted in the urine of a significant number of patients who had no symptoms of invasive candidiasis (95). Thus, although the diagnosis of a urinary tract infection should be considered in a patient having >10,000 *Candida* organisms per ml of urine, clinical correlation is essential in interpreting any urine culture positive for *Candida* spp. Some (65) have claimed that candiduria in a neutropenic leukemic patient is evidence of bloodborne dissemination. There is no evidence for this assertion, which is based mainly on studies in mice injected intravenously with *C. albicans* (66). In these animals, miliary abscesses develop in their kidneys and resolve slowly.

Diagnosis of *Candida* retinitis can present problems. This infection is bloodborne and begins as a lesion in the choroid layer of the retina, usually in an area in the posterior one-half of the eye. Growing organisms and necrotic debris project into the gelatinous vitreous humor overlying the retina to give the fluffy lesions seen on ophthalmoscopy (31). When an eye is surgically explored and *Candida* retinitis is the anticipated diagnosis, most surgeons at our institution submit a specimen taken from anteriorly located vitreous for Gram stain and culture. If yeasts are seen, thorough debridement is done and antifungal treatment is begun (89). If the Gram stain is negative, specimens of vitreous closer to the retina are submitted with the hope that a causative agent can be cultured, and the eye is closed. The vitreous is harvested with an instrument that provides continuous irrigation to the surgical field. Thus, it is common for the microbiology

laboratory to receive a specimen with a volume of up to 100 ml which contains only 0.1 to 0.2 ml of vitreous material. There are no published standardized techniques describing how to process such a specimen in the microbiology laboratory. I believe that a vitreal specimen should be concentrated by centrifugation or filtration and, if available, a cytocentrifuge-prepared slide should be submitted for Gram stain examination. Concentrates that are cultured should be inoculated into broth and onto an agar plate since finding multiple colonies on the plate increases confidence that yeast cells growing in the broth are not a contaminant.

Fungal peritonitis is a well-documented complication of chronic ambulatory peritoneal dialysis (32). It is important to distinguish between a contaminant introduced into the specimen from manipulation of the dialysis equipment and an organism actually resident in the fluid. For this reason, fluid should be spun and cultured both in broth and on plates. Some think that these specimens are so prone to contamination that a second specimen should be submitted when one is positive to verify that infection is truly present (4).

Cultures of Bronchoalveolar Lavage (BAL) Specimens

As discussed above, *Candida* colonization of the oropharynx is common, while pulmonary candidiasis is exceedingly rare. Therefore, reviews of pulmonary candidiasis have concluded that this diagnosis is made with certainty only by culturing *Candida* spp. from or demonstrating *Candida* spp. histologically in an open lung biopsy (70, 77, 97). These reviews were published prior to 1980. Since that time, there has been increasing interest in the use of bronchoscopy to diagnose pulmonary infections in immunocompromised patients. Transbronchial biopsy specimens cannot be obtained from all immunocompromised patients because many of them have coagulopathies. Bartlett et al. (8) found that most bronchoscopically obtained bronchial washings contained mainly local anesthetic that had been used to facilitate passage of the bronchoscope and were heavily contaminated with oropharyngeal flora. Obviously, bronchial washings are undesirable for use in diagnosing pulmonary candidiasis.

BAL is performed by wedging the tip of the bronchoscope into a segmental bronchus and instilling 50- to 60-ml volumes of normal saline through the bronchoscope. BAL material is retrieved by suction with a syringe; a total of 150 to 250 ml of saline is instilled to obtain a 50- to 100-ml BAL specimen. The BAL differs from bronchial washings in two respects. First, BAL samples alveolar as well as bronchial contents. Second, although some oropharyngeal contamination of the specimen may occur, such contamination will be greatly diluted by the volume of lavage used. BAL has become a popular technique for diagnosing pulmonary infections in immunosuppressed patients, and protocols for analyzing BAL specimens in the microbiology laboratory have been published (54).

Unfortunately, there are limited data bearing on the interpretation of *Candida* spp. isolated from a BAL specimen. Working independently, Thorpe et al. (118) and Kahn and Jones (53) performed quantitative cultures of BAL specimens from a total of 190 patients. Of 33 patients with bacterial pneumonia, 29 had $>10^5$ bacteria per ml of BAL specimen. They concluded that this was a reasonable cutoff value for use in diagnosing bacterial pneumonia by BAL. Kahn and Jones (53) also examined Giemsa-stained, cytocentrifuge-prepared slides of BAL specimens for squamous epithelial cells and concluded that specimens with <1% squamous cells had insignificant oropharyngeal contamination. It is unknown whether similar standards can be used to diagnose pulmonary candidiasis with BAL. Saito et al. (98) recently reported diagnosing pulmonary candidiasis in four patients with autopsy-proven pulmonary candidiasis by using BAL. Unfortunately, they did not indicate whether numerous yeasts were seen in Gram stains of the BAL or the number of *Candida* cells growing from the specimens.

Semiquantitative or Quantitative Cultures of Tissues

Authorities disagree on the value of quantitative or semiquantitative cultures of tissue specimens from burns or large open wounds. Semiquantitative methods that allow the laboratory to report $<10^4$ CFU/g, $>10^6$ CFU/g, and exact CFU counts for specimens growing 10^4 to 10^6 CFU/g of tissue have been described (15). Several investigators have concluded that finding $>10^5$ bacteria per g of tissue correlates with the presence of sepsis (64, 96). Others have found histologic evidence for bacterial invasion in only 36% of burn wound biopsies containing $>10^5$ bacteria per g of tissue (72). They think the burn or wound biopsy culture is valuable principally for establishing the predominant flora in the tissue.

Unfortunately, although up to 30% of burned patients will have *Candida* spp. cultured from at least one site (110), there are no published data enabling one to judge the value of biopsy cultures in predicting sepsis from *Candida* spp. Therefore, one must either assume that the same cutoff values established for predicting bacterial invasion apply to yeasts or that, when a yeast is recovered as the predominant organism from a biopsy, it must be recognized as the pathogen most likely to be invading tissues.

Cultures of Blood

Before discussing studies comparing different techniques for culturing *Candida* spp. from blood, two points regarding their limitations must be made. First, none of these studies describe in detail characteristics of the patients from whom positive cultures were obtained. Thus, it is impossible to determine whether blood culturing is more effective in detecting fungemia in one population of patients compared with another. At the University of Wisconsin Hospital in 1986, a *Candida* sp. grew from blood cultures taken from 41 patients. In most cases, more than one blood culture from each of these patients was positive, suggesting that fungemia was persistent in most of the patients. Three of the patients with positive cultures were on pediatric wards, 7 were on surgical wards, 7 were on hematology-oncology wards, and 24 were in intensive care units. Obviously, several factors could explain the high proportion of positive cultures from intensive care unit patients, including their having more organisms per milliliter of blood during fungemia, more persistent fungemia, a higher frequency of fungemia, and more frequent culturing. All of the explanations except the last one pertain to differences in the pathogenesis of candidiasis in the intensive care patients compared with the other patient populations; pathogenesis of candidiasis in patients on medical wards and intensive care units were discussed earlier. Second, none of the studies comparing blood culture techniques addresses how any particular technique should be applied to optimize detection of fungemia. Using a broth culture technique, Ness et al. (81) recently reported that 82% of neutropenic leukemic patients with invasive candidiasis had at least one blood culture positive for *Candida* spp. This contrasts sharply with other studies that used the same blood

TABLE 1. Studies comparing different blood culture techniques for ability to recover *Candida* spp.[a]

Blood culture technique[b]	Reference	Brand name[c]	Total blood specimens cultured	No. of cultures positive			Mean time (days) for detection of positive cultures
				All *Candida* spp.[d]	*C. albicans* or *C. tropicalis*	*C. glabrata*	
Lysis-centrifugation	30		3,335	21 (11)	20	0	1.8
SP broth, V		BD	3,335	15 (5)	14	1	3.7
Lysis-centrifugation	57	Isolator	7,000	85 (30)	41	27	1.9
C broth, V		BBL	7,000	61 (6)	31	19	2.9
Lysis-centrifugation	12	Isolator	11,000	76 (24)		7	
TS broth, A		BACTEC	11,000	64 (12)		2	
Lysis-centrifugation	11	Isolator	2,188	68	56	3	1.9
TS broth, A		BACTEC	2,188	56	47	2	2.7
Lysis-centrifugation	13	Isolator	5,000	25 (10)			
TS R broth, A		BACTEC	5,000	19 (4)			
Lysis-centrifugation	10	Isolator	5,125	64 (24)	34	24	2.3
Biphasic, BHI, V		BBL	5,125	49 (9)	24	20	5.0
Lysis-centrifugation	43	Isolator	23,586	164 (70)	127	25	1.9
Biphasic, BHI, V		Septi-Chek	23,586	115 (21)	90	15	4.4
Lysis-centrifugation	38	Isolator	3,111	26 (12)	25	1	
Lysis-filtration			3,111	24 (10)	23	1	
Biphasic, BHI, V	56	GIBCO	5,000	29	18	3	
BHI broth, V		GIBCO	5,000	27	19	1	
Biphasic, TS, V	124	GIBCO	3,537	27 (1)			2.3
TS broth, V		GIBCO	3,537	30 (4)			3.0
Biphasic, BHI, V	92	BBL	668	24	17	2	7.9
TS broth, A		BACTEC	38,324	147	108	7	2.0
Biphasic, TS, V	79	Septi-Chek	5,034	39 (28)	14	25	
TG broth, NV		Signal	5,034	22 (0)	4	18	

[a] All studies except that for reference 92 were done with blood samples that were split and cultured simultaneously, using the techniques shown. Data for reference 92 are shown because it contrasted the time required for detecting a positive culture by the two techniques used in this study. In a study related to reference 79, investigators found that, when Signal and BACTEC culture bottles were inoculated with *C. albicans*, BACTEC cultures had detectable growth 24 h prior to Signal cultures (119). In another related study comparing isolator and BACTEC techniques for recovery of bacteria (20), investigators classified five isolations of *Candida* spp. which were from Isolator cultures but not from BACTEC cultures as contaminants.

[b] SP, Supplemented peptone; TS, tryptic soy; C, Columbia; BHI, brain heart infusion; TG, thioglycolate; A, aerobic; V, vented; NV, not vented; R, resin.

[c] Manufacturers of culture devices: Isolator, Du Pont de Nemours & Co., Inc., Wilmington, Del.; BACTEC, Becton Dickinson Diagnostic Instruments, Towson, Md.; BBL, BBL Microbiology Systems, Cockeysville, Md.; Septi-Chek, Roche Diagnostics, Div. Hoffmann-La Roche Inc., Nutley, N.J.; Signal, Oxoid USA, Inc., Columbia, Md.; GIBCO, Life Technologies, Inc., North Andover, Mass.; BD, Becton Dickinson and Co., Rutherford, N.J.

[d] Number in parentheses refers to number of positive cultures obtained only by the corresponding technique.

culture technique in which only 25 to 29% of such patients had a culture positive for *Candida* spp. (40, 73). This striking difference is probably explained by the fact that Ness et al. obtained blood cultures nearly daily from each patient in their study (an average of 39 cultures per patient admission). If *Candida* fungemia were only transient in these patients and only small numbers of organisms were present during fungemia, frequency of culturing and total volume of blood cultured during an admission might influence the likelihood of detecting fungemia more than the particular blood culture technique used.

Studies comparing different blood culture techniques for sensitivity in detecting *Candida* spp. in the blood are summarized in Table 1. The data indicate that the lysis-centrifugation technique (55) is more sensitive than any of the other techniques tested. Lysis-centrifugation and lysis-filtration techniques have about the same diagnostic yield. Biphasic media appear slightly better than or equivalent to broth in recovering yeasts. Growth appears to be detected about 1 day earlier in lysis-centrifugation cultures than in broth cultures. The big delay in detecting growth in biphasic media seems to be inherent in their use. Isolator culture tubes (E. I. duPont de Nemours & Co., Inc., Wilmington, Del.) are available commercially for performing the lysis-centrifugation technique. Because BACTEC broth culture bottles (Becton Dickinson Diagnostic Instruments, Towson, Md.) can be screened automatically by radiometric or spectrographic methods, they are used by many large microbiology laboratories.

Lysis-centrifugation cultures have the potential advantage of enabling one to estimate the number of CFU of an organism present per milliliter of blood. Whimbey et al. (127) recently described two patients with hematologic malignancies in whom blood cultures drawn from Broviac or Hickman catheters grew 14 to >100 CFU of *Candida* spp. per ml, whereas cultures drawn from peripheral veins had only 0.1 to 0.2 CFU/ml. Thus, they thought that the lysis-centrifugation technique enabled diagnosis of fungemia related to an

infected intravascular device. In a conflicting study, Paya et al. (88) cultured blood simultaneously from a peripheral vein and intravascular devices by using the lysis-centrifugation technique during septic episodes in 44 patients. Of 52 intravascular cannulae studied, 15 were the cause of bacteremia or fungemia but only 7 of these showed a significantly higher bacterial count in blood obtained through the device as compared with peripheral blood. In the one patient with sepsis due to *C. albicans*, both blood specimens showed <10 CFU/ml. Bacterial counts were higher in blood drawn through the device than in peripheral blood in four of six cases that did not fulfill their definition of intravascular device-related bacteremia. Thus, data regarding the usefulness of lysis-centrifugation cultures in diagnosing intravascular device-related fungemia are conflicting and very limited.

Several studies have concluded that the Isolator is more sensitive in detecting fungi other than *Candida* spp. in blood (10, 12, 43). However, setting up an Isolator culture in the microbiology laboratory requires more time than placing BACTEC bottles in the automated screening instrument. Individual laboratories need to decide whether the greater sensitivity of the lysis-centrifugation technique in detecting fungi warrants its use for routine blood culturing. Depending on the patient populations in its hospital and work load, a laboratory may choose to use only broth cultures, to use broth cultures routinely and lysis-centrifugation only when fungal cultures are specifically ordered by clinicians, or to use lysis-centrifugation as the routine blood culturing technique.

Cultures of Intravascular Devices

In a febrile patient with a complex clinical course, cultures of intravascular catheter tips can be important in determining whether the catheter insertion site is infected and acting as a portal of entry for an organism. Quantitative culturing is essential to distinguish between contaminants picked up from the skin when the catheter is removed and actual colonization of the catheter tip. Unfortunately, the cutoff values for discriminating infection from contamination have been well established for bacteria but not for *Candida* spp. At present, one must use these cutoffs as a guide in interpreting cultures that grow yeasts. In the technique of Maki et al. (69), the catheter is rolled over the surface of an agar plate; >15 CFU of an organism has been statistically correlated with localized inflammation or systemic infection. Brun-Buisson et al. (14) recommend a more traditional quantitative technique. The catheter segment is sectioned and placed in a tube containing 1 ml of sterile water. The tube is mixed on a Vortex mixer for 1 min and 0.1 ml is spread over an agar plate with a sterile Pasteur pipette which has been bent into a U shape. They found that counts of $\geq 10^3$ CFU bacteria per ml were 97.5% sensitive and 88% specific for a catheter-related infection. Their study included only one case of *C. albicans* catheter sepsis, and the catheter grew 3.8×10^2 CFU/ml.

DIAGNOSTIC METHODS OTHER THAN CULTURES

Fluorescence Microscopy to Detect Organisms in Specimens

Reagents for direct or indirect fluorescent-antibody staining of *Candida* species are not available commercially. However, rabbit serum with a high antibody titer to *C. albicans* will recognize all *Candida* species when used in an indirect fluorescent-antibody stain of infected tissues. We have found fluorescent-antibody staining useful in the diagnosis of *Candida* hepatitis (unpublished observations). As described, the liver is efficient in killing *Candida* spp.; therefore, when only a few millimeters of biopsy tissue is cultured, the organism may not be recovered. The yeast can be difficult to find in histologic sections. The density of organisms in the tissue may be low, and they will be missed unless multiple sections are examined. Also, the organism may be present in necrotic material which will detach from the specimen and not be processed by histologic techniques. A fluorescent-antibody-stained smear prepared from a homogenate of a portion of the biopsy specimen can help diagnose the infection when both cultures and histologic examinations are negative (Fig. 1).

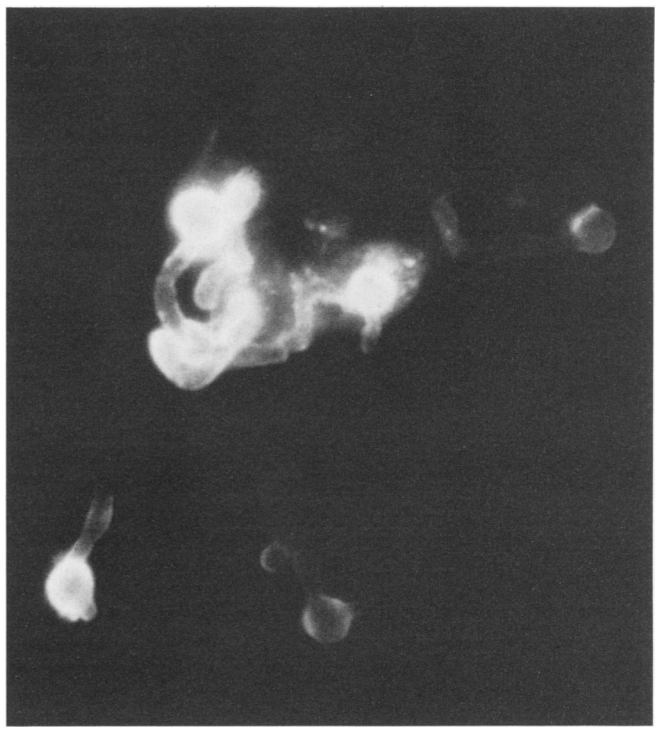

FIG. 1. Immunofluorescent staining of *Candida* organisms. A needle biopsy of a granulomatous lesion from the liver of a patient with acute leukemia was quite friable. Several tiny particles of the biopsy fell into the saline solution in which it was originally placed. These were homogenized in 0.1 ml of saline and applied to slides. After air drying, the slides were fixed in ethanol and stained with fluorescein-labeled rabbit anti-*Candida* antibodies. Several fields showed blastospores with attached germ tubes (original magnification, ×1,000). The remainder of the biopsy, which was submitted for histologic examination, showed what appeared to be the edges of an inflammatory lesion. Presumably, the organisms were present in the necrotic material that was stained by the fluorescent antibodies. A second biopsy submitted for culture failed to grow any organisms.

Zufferey et al. (131) recently described using an acridine orange stain to detect microorganisms adherent to catheters submitted for culture. Each catheter was first cultured semiquantitatively by Maki's technique (69) and then fixed and stained with acridine orange. Fixation and staining required 6 min. The stained catheter was placed on a microscope slide and examined for 3 min, using an epifluorescent microscope. This type of illumination is essential because the UV light beam was directed over the surface of the catheter rather than from below the stage. They found

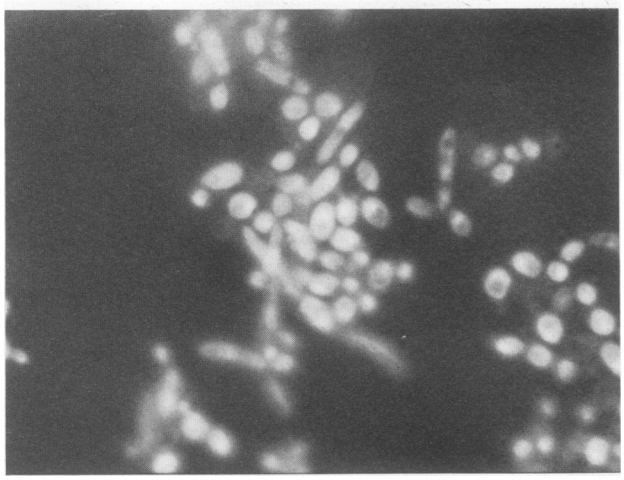

FIG. 2. Catheter colonized with *C. tropicalis* (original magnification, ×1,000). Yeast cells appear as large oval blastospores with various degrees of staining with acridine orange. The catheter had been cultured by rolling it over an agar plate prior to staining with acridine orange and examination on the stage of a microscope equipped for epifluorescence. Reproduced from reference 131 with permission of the publisher.

that stained microbes could be seen on 84% of catheters that grew ≥15 CFU. Yeast cells were readily detected on catheters by this technique (Fig. 2). If a laboratory received a large number of catheter tips for culture, staining them all with acridine orange would require a significant amount of technician time. However, the procedure would provide the clinician with valuable information approximately 24 h before a culture result would be available.

Detection of Anti-*Candida* Antibodies in Sera

Because of difficulties in distinguishing *Candida* colonization from invasion and the low rate of positive blood cultures in patients with invasive disease, there has been keen interest in the serodiagnosis of invasive candidiasis. A voluminous literature describes methods for detecting anti-*Candida* antibodies in human sera (1, 2, 5, 21, 24, 25, 40, 41, 44, 46–49, 58, 59, 71, 73, 81, 90, 91, 111, 113, 116, 117). Methods range in sophistication from detection of precipitins against crude extracts of *C. albicans* to quantitation of antibodies against specific antigens by radioimmunoassays (RIA) or enzyme immunoassays (EIA). When one reviews all of the published studies describing antibody detection, a few general conclusions are possible. Most studies published between 1957 and 1978 used poorly characterized antigenic extracts of *C. albicans* for precipitin testing or passive hemagglutination assays (1, 2, 5, 25, 44, 46, 47, 59, 91, 111, 113, 117). In some, agglutination of *C. albicans* blastospores was used as the assay for antibodies (46, 91). In many of these studies, remarkably high test sensitivities and specificities were claimed (25, 91, 117). The studies were flawed in several ways. The crude antigen preparations used in different laboratories could not be standardized to enable good test reproducibility among laboratories. Although the positive precipitin tests obtained with a yeast cytoplasmic extract were said to enable one to discriminate between tissue invasion and colonization with *Candida* spp. because they detected antibodies against internal antigens of the microorganism, definitive proof of this was not obtained (59). More recent work has shown that cytoplasmic extracts contain appreciable amounts of cell wall mannan (CWM), a polysaccharide antigen abundant in *Candida* cell walls (48, 49). In addition, these earlier studies were biased because sera from patients with severe invasive candidiasis were often used to assess the sensitivity of the assays whereas their specificity was determined with sera from healthy volunteers as controls (1, 2, 5, 25, 46, 111, 113, 117).

After 1980, attention was directed towards using defined purified antigens in the immunoassays and assessing their sensitivity and specificity in a more rigorous way. CWM of *C. albicans* shares antigenic determinants with CWM from other *Candida* species, and large amounts of highly purified CWM could be readily obtained. By using RIA and EIA, anti-CWM antibodies (anti-CWM) have been shown to be ubiquitous in human sera (21, 40, 48, 49, 58, 73). Anti-CWM appears to be one of the major antibody species in human sera. In any defined population, anti-CWM levels are usually distributed about a mean; sera having the highest levels give positive precipitin tests when tested against CWM (49). When anti-CWM levels were measured in serially drawn sera from neutropenic patients, a frequency distribution plot showed that antibodies from patients with invasive candidiasis were elevated and tended to skew the normal distribution curve to the right; however, a clear bimodal distribution of anti-CWM values was not observed (40). Therefore, to use anti-CWM measurements diagnostically, it was necessary to establish a cutoff value for anti-CWM levels that gave acceptable test sensitivity and specificity. When this was done, the best observed sensitivity was about 65% (40). In a significant number of patients, the test did not become positive until after a tissue biopsy diagnosis was made.

Counterimmunoelectrophoresis has been used to detect antibodies against multiple components of a cytoplasmic extract; the method is two- to fourfold more sensitive than conventional agar gel precipitin testing (24). Sera from patients with invasive candidiasis yielded more precipitin lines by counterimmunoelectrophoresis than sera from uninfected controls (5, 24). Cytoplasmic extracts were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and Western blots (immunoblots) of the resultant gels were probed with sera from infected and uninfected patients. This analysis revealed immunodominant cytoplasmic protein antigens that had molecular weights of 40,000 to 60,000 (41, 49, 71, 116). When CWM-free preparations enriched for these antigens were used as targets in RIA or EIA, antibodies against them could be detected in 25 to 70% of sera from neutropenic patients with invasive candidiasis.

Thus, to measure anti-*Candida* antibodies reliably, one must perform assays that are relatively time-consuming for which neither the requisite purified antigens nor test kits are commercially available. Antibody testing is unlikely to be useful in highly immunosuppressed patients, such as neutropenic bone marrow transplant recipients, who are at greatest risk for invasive candidiasis. In other patient populations, such as intensive care patients, who appear to mount antibody responses more reliably, it is not clear that performing an antibody assay adds much to the data base of accumulated culture reports that a clinician would use in making therapeutic decisions.

Detection of *Candida* Antigens in Sera

Since 1976, several investigators have reported attempts to develop immunoassays for detecting *C. albicans* antigens in sera from infected patients (3, 6, 9, 17, 19, 26, 28, 35–37, 42, 52, 62, 73, 75, 81, 102, 112, 123, 125, 126). The simple

TABLE 2. Immunoassays for detection of *Candida* antigens[a]

Assay method (reference)[b]	Source of detecting antibody[c]	Pretreatment of serum	Antigen used to standardize assay	Patient population[d]	Percentage of patients having positive assay result (total no. of patients)	
					Proven invasive candidiasis	Controls
HAI (126)	Rab-WCE-Adj-i.m., Rab-B-Adj-i.m.	No	Mannan	Mixed	29 (14)	1 (282)
EIA-D (123)	Rab-WCE-Adj-i.m.	No	None	Mixed	100 (3)	None
RIA-I (125)	Rab-WCE-Adj-i.m.	Acid-heat	Mannan	Mixed	47 (15)	0 (40)
EIA-I (102)	Rab-B-i.v.	Base	Mannan	Mixed	100 (7)	0 (20)
RIA-D (112)	Rab-WCE-Adj-i.m.	No	WCE	Mixed	55 (22)	0 (23)
HAI (75)	Rab-WCE-i.v./i.m.	No	WCE	Leuk/lymph	59 (32)	6 (71)
EIA-I (73)	Human serum	Base	Mannan	Leuk	100 (7)	9 (82)
EIA-D (62)	Rab-B-i.v.	Protease-heat	Mannan	Mixed	53 (15)	7 (449)
EIA-D (3)	Rab-WCE-i.m.	No	WCE	Leuk	100 (7)	0 (53)
EIA-D (28)	Rab-B-i.v.	EDTA-heat	Mannan	Leuk/lymph	70 (23)	0 (65)
EIA-D (35)	Rab-B-Adj-i.m.	EDTA-heat	Mannan	Leuk/lymph	100 (7)	4 (70)
LA (17)	Rab-WCE-Adj-i.m.	No	WCE	Leuk/lymph	90 (30)	10
				Mixed	100 (61)	11
LA (6)	Rab-B-i.v.	Protease-heat	Mannan	Leuk	65 (31)	2 (48)
LA (52)	Rab-B-i.v.	Protease-heat	Mannan	Leuk	78 (23)	16 (74)
				Mixed	22 (32)	6 (105)
LA (37)	RAB-B-Adj-i.m.	No	None	Mixed	91 (33)	2 (107)
LA (19)	RAB-B-Adj-i.m.	No	None	Mixed	67 (30)	5 (400)
LA (36)	RAB-B-Adj-i.m.	No	None	Mixed	71 (24)	4 (24)
LA (26)	RAB-B-Adj-i.m.	No	None	Mixed	94 (36)	0 (16)
LA (6)	RAB-B-Adj-i.m.	No	None	Leuk	23 (22)	0 (25)
LA (52)	RAB-B-Adj-i.m.	No	None	Leuk	48 (23)	11 (74)
				Mixed	19 (32)	11 (105)
LA (81)	RAB-B-Adj-i.m.	No	None	Leuk	55 (11)	34 (206)

[a] The last seven studies deal with use of the commercially available CAND-TEC test (Ramco Laboratories Inc., Houston, Tex.). It should be noted that, in addition to the differences shown, the two LA tests described in the two studies preceding these in the table (references 6 and 52) used beads sensitized with an IgG fraction, whereas the CAND-TEC beads (based on reference 37) are sensitized with whole serum. In the LA study described in reference 17 it was not possible to determine the number of controls used to calculate the percentages shown.

[b] HAI, hemagglutination inhibition; EIA-D, direct-sandwich EIA; EIA-I, antigen inhibition EIA; RIA-D, direct-sandwich RIA; RIA-I, antigen inhibition RIA.

[c] Rab, rabbit; WCE, whole-cell extract; B, blastospores; Adj, adjuvant; i.m., intramuscularly; i.v., intravenously.

[d] Leuk, Neutropenic leukemics; lymph, lymphoma patients; mixed, mixture of underlying diagnoses.

latex agglutination (LA) tests for detecting *Cryptococcus neoformans* polysaccharide in cerebrospinal fluid had proved extraordinarily useful in diagnosing cryptococcal meningitis, and it was thought that a similar assay for detecting a *Candida* antigen in sera from patients with invasive candidiasis could be developed. Table 2 summarizes studies that have been done with the most sensitive assays, RIA, EIA, and LA. Work done with counterimmunoelectrophoresis is not tabulated because it is a less sensitive technique. The assays for antigens are of three types: the first (6, 28, 35, 52, 62, 73, 125, 126) is thought to detect CWM; the second (3, 17, 75, 112, 123), to detect *Candida* antigens that are not CWM; and the third, to detect an antigen that has not been characterized (6, 19, 26, 36, 37, 52, 81).

Soon after the initial report describing detection of CWM in a small number of patients with invasive candidiasis (126), it became evident that anti-CWM and other CWM-binding proteins are present in human sera which prevent detection of this antigen in nearly all patients (49, 125). Therefore, detection of CWM is possible only when sera are treated to destroy these binding proteins before testing for CWM. Addition of acid, base, or proteolytic enzyme, followed by heating or boiling and then centrifugation, have been used to accomplish this goal (6, 28, 35, 52, 62, 73, 102, 125). The resultant supernatant is assayed for antigen.

In experimental animal models of candidiasis and in studies of infected humans, it became evident that CWM is cleared rapidly from the serum (48). Even in severe infections, CWM levels rarely reach 100 ng/ml (48). Because anti-CWM antibodies raised in rabbits or obtained from mouse hybridomas have low avidity for CWM, assay systems have been used to detect antigen levels near the threshold of their sensitivities. Not surprisingly, detection of CWM is specific for the diagnosis of invasive candidiasis; however, reported diagnostic sensitivities have ranged between 22 and 100%. The lowest diagnostic sensitivities have been seen in patients from whom only one or two serum samples were assayed at the time infection was suspected. The best sensitivities were seen in neutropenic patients from whom serial specimens had been obtained weekly. The three largest studies (6, 52, 73) that used sequential serum assays had diagnostic sensitivities of 65 to 100%. These data suggest that diagnostic sensitivity is about 70% when sera from patients at risk for developing invasive candidiasis are assayed weekly. Because of the inconvenience associated with sample preparation and the difficulties associated with using immunoassays near the threshold of their sensitivities, commercial preparation of a CWM detection kit has not been possible.

Assays thought to detect antigens other than CWM have been developed that use rabbit antibodies raised by intramuscular injection of whole-cell extracts of *C. albicans* in an adjuvant. The ability of an assay to detect *Candida* antigens is verified by noting whether the antigen preparation used for immunization can be detected. The immunization procedure

favors production of antiprotein antibodies. Also, because sera are not pretreated before the assay is done, detection of CWM would be very unlikely. The sensitivity of these assays has ranged from 55 to 100%. Each laboratory has used a different type of assay system (hemagglutination inhibition, EIA, RIA, or LA), so it is difficult to make comparisons. Although monoclonal antibodies against cytoplasmic antigens have been produced (115), their successful application in an immunoassay to detect antigens in human sera has not been described.

The only commercially available antigen detection assay for use in diagnosing candidiasis is an LA test (CAND-TEC; Ramco Laboratories Inc., Houston, Tex.), which is based on work described by Gentry et al. in 1983 (37). Latex particles are sensitized with sera from rabbits immunized intramuscularly with a heat-killed suspension of *C. albicans* blastospores. The nature of the antigen detected by this assay has not been determined. We could not produce agglutination of CAND-TEC beads with either purified CWM or a whole-cell cytoplasmic extract of *C. albicans* at concentrations ranging from 1 ng to 1 mg/ml (unpublished observations). In a rat model of disseminated candidiasis, Greenfield et al. (42) detected either CWM by EIA or mannose by high-pressure liquid chromatography, but the CAND-TEC test applied to the same sera was uniformly negative. Therefore, this test may detect a neoantigen of *C. albicans* which requires some action of human host cells on the organism before it is detected. Alternatively, the antigen could be some material released from the human host during invasive candidiasis which happens to be detected by antibodies stimulated by heat-killed *C. albicans* in a rabbit.

The usefulness of the CAND-TEC assay for diagnosis of invasive candidiasis in patients with a variety of underlying diseases has been explored in four studies (19, 36, 37, 52). The authors reported diagnostic sensitivities between 19 and 91% and specificities between 89 and 95%. In each of these studies, different criteria for diagnosing invasive candidiasis were used, and in many instances the descriptions of patients with invasive candidiasis were too limited to confirm that they had invasive infection rather than colonization or superficial infection by *Candida* spp. Also, in three studies (19, 36, 37), the control sera tested were from either healthy volunteers or patients who were not clearly matched with the infected patients for underlying disease. These problems make these studies different to interpret.

In three studies focusing on use of the CAND-TEC test in patients with acute leukemia (6, 52, 81), its diagnostic sensitivity ranged between 23 and 55%, while specificity ranged between 66 and 100%. Diagnostic sensitivity tended to increase as the number of sera tested per patient increased. However, as the number of sera per patient increased, the specificity also fell appreciably. Ness et al. (81) studied 2,181 sera from 217 patient admissions complicated by neutropenia. Approximately two sera were collected weekly from each patient. Of 41 patients who died and had no evidence of candidiasis at autopsy, 29 (71%) had a positive test result. Two-thirds of these patients had received amphotericin B treatment, but some evidence supporting *Candida* infection would have been expected at postmortem. Therefore, Ness et al. may have been observing false-positive tests. Interestingly, patients with positive tests were statistically more likely to have a creatinine level of >2 mg/dl, and in some cases antigen titers paralleled a rise in serum creatinine. Their data suggest that the serum level of either *C. albicans* antigen or some other factor capable of producing a positive test result was related directly or indirectly to the presence of renal failure.

At present, no commercially available test for detection of *Candida* antigens can be recommended for use. It does not seem likely that a test kit for detecting CWM will be marketed soon. Of the assays described, the LA tests are most promising because of their simplicity. Any test for detecting a *Candida* antigen that is marketed commercially should be simple to perform and not require any pretreatment of serum. Also, because sera need to be tested frequently, the cost per test would have to be low.

Detection of Metabolites of *Candida* spp. in Sera

Mannose and arabinitol are metabolites of *Candida* spp. which can be readily detected in sera by gas-liquid chromatography. By using suitable internal standards, serum concentrations of the metabolites can be determined. The techniques used and much of the pertinent literature have been reviewed by de Repentigny and Reiss (29). There are many technical issues that must be considered in both performing the chromatography and interpreting the results. Sugars must be separated chemically from serum and derivatized prior to chromatography. Workers disagree about the derivatives that should be prepared (28, 29, 129; B. Wong, E. M. Bernard, D. Armstrong, J. Roboz, R. Suzuki, and J. F. Holland, letter, J. Clin. Microbiol. **21**:478–479, 1985). When serum is hydrolyzed prior to derivatization, mannose from host serum glycoproteins as well as mannose produced by the fungus will be detected. Healthy humans have detectable serum levels of free mannose and arabinitol; mannose is a normal product of human metabolism. Also, *Candida* and other microbes colonizing humans could release mannose or arabinitol, contributing to the serum levels measured. Changes in normal flora of patients receiving chemotherapy or antibacterial drugs could influence the concentrations of mannose and arabinitol detected in their sera. Serum mannose levels can be elevated in diabetic patients with ketoacidosis. Rabbits given corticosteroids have elevated concentrations of mannose and, presumably, so would humans given steroids (27). Finally, arabinitol is cleared by the kidneys with approximately the same efficiency as creatinine. Patients with renal failure will have higher arabinitol levels and the ratio of arabinitol to creatinine must be used to interpret any observed concentration of arabinitol (39, 130).

Comparisons between metabolite determinations and antigen detection for the diagnosis of invasive candidiasis have been made in only one study (28). Sera from 50 healthy blood donors and 38 high-risk patients, 23 with and 15 without invasive candidiasis, were analyzed by gas-liquid chromatography for arabinitol and mannose content and by EIA for detectable CWM. Arabinitol and mannose levels and the arabinitol/creatinine ratios were significantly higher in the high-risk patients without candidiasis than in healthy blood donors. Diagnostic utility (sensitivity/specificity) was as follows: for elevated arabinitol, 26/87%; for elevated arabinitol/creatinine ratio, 13/93%; for elevated mannose, 39/87%; and for CWM detection, 65/100%.

Aside from the above considerations, the greatest drawbacks to gas-liquid chromatography are the long time required to perform an assay and the complexity of the equipment used. Only a single specimen can be assayed at a time, and perhaps only six to eight specimens could be assayed in a working day.

Typing *C. albicans* Strains and Use of DNA Probes Derived from *C. albicans*

For years it has been assumed that, when a patient becomes infected with *C. albicans*, the infecting strain is one with which the patient has been chronically colonized. Until recently, systems for typing *C. albicans* isolates were not available, so this hypothesis could not be challenged. Routine sugar assimilation or fermentation tests cannot distinguish strains, and only two CWM serotypes were recognized. Phenotypic and genotypic methods are now available for typing *C. albicans*.

Since *C. albicans* is a diploid eucaryote, recessive and dominant gene expression is possible and stable nonlethal mutations in a single cell can be passed on to its progeny. Therefore, different phenotypes can be observed when appropriate observations are made. Two approaches to phenotypic typing have been attempted. The first involves noting the growth characteristics of isolates replica plated onto different indicator or selective media. Patterns of growth characteristics on the different media are coded to yield biotypes (83, 84) or resistograms (122). Only a few laboratories have used these typing techniques because preparation and reading of the plates require great care. Also, although >500 biotypes are hypothetically possible, biotypes cluster into about 20 groups with similar phenotypes (85). Odds et al. (86) recently reported using a biotyping method to type 18 coded *C. albicans* isolates in four laboratories. The biotyping system gave excellent intralaboratory reproducibility; however, concordance of data among laboratories was poor. A related typing technique has been described by Slutsky et al. (103) and Soll et al. (104, 105). When a single colony of an isolate is plated onto an amino acid-rich defined medium and incubated at 24°C, a variety of distinct colony morphologies is seen, each occurring at a particular frequency. When colonies on this plate are picked and replated, a pattern of different colony morphologies is again seen. A rapid genetic switching mechanism is responsible for the varying colony morphology. Isolates differ in the repertoire of colony morphologies to which they can switch, and the observed repertoire itself amounts to a phenotype. Obviously, this typing method is very laborious and is useful mainly as a tool for genetic studies of the fungus. In the second approach to phenotyping, whole-cell extracts of isolates are applied to sodium dodecyl sulfate-polyacrylamide gels and the patterns of protein bands are compared (71, 120).

Several approaches have been used for genotyping *C. albicans*. In the first, whole-cell DNA extracts of isolates are digested with a restriction endonuclease, usually a tetraschizomer. Digests are separated electrophoretically in agar gels, and the restriction fragment length patterns (RFLPs) are read directly (100, 120). Alternatively, Southern blots of the gels are probed with cloned fragments of a representative strain (34, 67, 101, 104, 105). The cloned fragments are generally of relatively large size and are repeated within the genomic DNA. The number of RFLPs recognizable by inspection of agar gels is limited; however, when probes are used, very large numbers of RFLPs can be seen on blots. In some instances, the RFLP obtained from an isolate that has been passaged multiple times in the laboratory differs from that seen when the microbe was originally isolated (101). Presumably, this is due to mutations occurring during multiple rounds of cell division.

C. albicans isolates have been typed by their mitochondrial DNA (mtDNA) polymorphism (87). This technique is promising because each blastospore contains numerous copies of mtDNA, guaranteeing that a strong signal is obtained in probed blots. Also, studies in higher organisms have indicated that variations in mtDNA can be quite stable and can be used as clonal markers to recognize descendants of a particular cell. However, Fox et al. (34), using the published technique for typing by mtDNA polymorphism (87) to analyze a group of isolates, concluded that it did not yield a large enough number of types to be useful in epidemiologic studies. W. S. Riggsby's laboratory and our laboratory have collaborated in developing a refinement of a typing scheme for *C. albicans* based on mtDNA polymorphism. Five *Eco*RI fragments of mtDNA from a laboratory strain of *C. albicans* were cloned. We found that, when blots of *Msp*I digests of whole-cell DNA extracts that had been resolved on agar gels were probed with the cloned *Eco*RI mtDNA fragment E2, RFLPs corresponding to 11 types of *C. albicans* could be readily demonstrated (unpublished observations). The types were stable with passage of isolates in the laboratory, and the typing technique was used to document spread of a particular type among patients in an intensive care unit.

Studies that use biotypes or typing by different protein migration in sodium dodecyl sulfate-polyacrylamide gels, restriction endonuclease digest fragment patterns of whole-cell DNA, and mtDNA polymorphism have all indicated that there are different strains of *C. albicans* and that *C. albicans* can be spread from patient to patient in intensive care units (18, 71, 120). Evidence points to hands of hospital personnel as the vector for transferring *C. albicans* between patients (18). The typing methods have shown that patients can be infected with a *C. albicans* strain that is part of their normal flora or with a nosocomially acquired strain. In spite of their utility, all of the typing methods are laborious to perform, and it is likely that they will be used only as research tools or to track outbreaks of hospital-acquired candidiasis in centers treating large numbers of immunocompromised patients.

A DNA fragment has been cloned from a *C. albicans* isolate which is capable of detecting DNA of several *C. albicans* isolates or a representative isolate of *C. stellatoidea* on nitrocellulose blots. However, it did not hybridize with DNA from other fungi or murine or human cells (23). The role of such a cloned fragment in detecting *Candida* spp. in tissues or specimens or in clarifying taxonomy of these species remains to be determined.

SUMMARY: PRESENT AND FUTURE ROLES OF THE MICROBIOLOGY LABORATORY

At the present time, the decision to treat a patient for invasive candidiasis is based mainly on an analysis of patient risk factors, known pathogenetic mechanisms for invasive candidiasis, and the results of cultures taken from the patient. Therefore, the role of the microbiology laboratory is a rather traditional one. It is important that, when cultures are submitted from an immunocompromised patient, isolation of *Candida* species are reported as quickly as possible. Even when part of a complex flora, *Candida* spp. isolated from the peritoneal cavity should be reported. The lysis-centrifugation culture technique is more sensitive than other methods in detecting *Candida* spp. in blood; growth of *Candida* spp. from cultured blood is also detected sooner by this technique. As discussed earlier, individual laboratories will have to weigh a number of factors in deciding whether lysis-centrifugation should be used as a routine blood culture technique or only when the clinician orders a fungal blood culture. Regardless of the blood culture technique used, recent work (81) suggests that, if clinicians obtain blood

cultures frequently from neutropenic patients, *Candida* fungemia may be detected often in patients developing invasive candidiasis.

Unfortunately, studies have not been done that enable one to correlate the result of a quantitative intravascular catheter culture that grows a *Candida* sp. with the presence or absence of infection at the site from which the catheter was removed. Presently, one must rely on cutoff values established for bacterial infections to interpret such cultures that grow *Candida* spp. Authors disagree about the value of quantitative or semiquantitative tissue biopsies for detecting tissue invasion by bacteria. Very little data about the interpretation of growth of yeasts from such cultures are available.

Although a good deal of research has been directed at developing diagnostic tests to detect anti-*Candida* antibodies or *Candida* antigens in sera, it has not been possible to develop tests with requisite simplicity, sensitivity, and specificity for the clinical microbiology laboratory. Before detection of metabolites of *Candida* spp. in sera by gas-liquid chromatography could be used in the microbiology laboratory, technical issues related to derivatization of the metabolites must be resolved. The time required to process specimens and the complexity of the instruments required for high-pressure liquid chromatography analysis will make practical application of this technology difficult.

The ability to type isolates of *C. albicans* has already led to interesting observations regarding its transmission within the hospital; however, it seems unlikely that any of the typing techniques presently available will be used as anything but research tools for the forseeable future.

The future role of the microbiology laboratory in diagnosis of invasive candidiasis could be influenced by research directed at answering a number of questions. For example, how does drawing blood cultures frequently, on a scheduled basis, from patients at high risk for developing candidiasis (e.g., neutropenic patients with leukemia or intensive care unit patients) influence the likelihood of detecting *Candida* fungemia compared with an approach in which blood cultures are drawn only when infection is clinically suspected? If scheduled blood cultures are drawn, do lysis-centrifugation cultures detect more episodes of fungemia than broth cultures? Can one establish cutoff values for interpreting growth of *Candida* spp. from quantitative cultures, tissue biopsies, or BAL specimens? Can a *C. albicans* antigen be found that is released during tissue invasion but fails to bind to anti-*Candida* antibodies or other serum proteins so that it can be detected in serum that was not pretreated to destroy antibodies? It is hoped that answers to these questions will be forthcoming during the next several years.

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