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Simplified Adsorption Method for Detection of Antibodies to *Candida albicans* Germ Tubes

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Two modifications that simplify and shorten a method for adsorption of the antibodies against the antigens expressed on both blastospore and germ tube cell wall surfaces (methods 2 and 3) were compared with the original method of adsorption (method 1) to detect anti-*Candida albicans* germ tube antibodies in 154 serum specimens. Adsorption of the sera by both modified methods resulted in titers very similar to those obtained by the original method. Only 5.2% of serum specimens tested by method 2 and 5.8% of serum specimens tested by method 3 presented greater than one dilution discrepancies in the titers with respect to the titer observed by method 1. When a test based on method 2 was evaluated with sera from patients with invasive candidiasis, the best discriminatory results (sensitivity, 84.6%; specificity, 87.9%; positive predictive value, 75.9%; negative predictive value, 92.7%; efficiency, 86.9%) were obtained when a titer of ≥1:160 was considered positive.

Invasive *Candida* infections are a growing problem among patients put at risk by advances in medical technology, chemotherapy, cancer therapy, or organ transplantation. The management of serious and life-threatening candidiases remains severely hampered by the lack of both specific clinical manifestations and the accuracy of conventional microbiological methods for diagnosis (2). These problems have led to demands for the development of reliable serological tests for the diagnosis of invasive *Candida* infections. Among the characteristics of such tests, relative simplicity may be one of the most important, since it will facilitate their adoption by clinical microbiology laboratories. Detection of anti-*Candida albicans* germ tube antibodies (anti-CAGTA) by an indirect immunofluorescence assay, which has been shown to be useful in the serodiagnosis of invasive candidiasis in humans and animals (6–9), may accomplish this goal, since indirect immunofluorescence technology is available in most clinical laboratories. However, detection of anti-CAGTA requires four adsorptions of the sera, a process that usually takes 24 h and complicates this test. Adsorption of the sera removes antibodies that react with antigens expressed on both blastospore and germ tube cell wall surfaces and confers specificity on the test (6). Newer methods for *Candida* serology are mainly based on detection of antigens (1, 4). However, detection of antibodies may be useful in some patients. In this report, we present two modifications of the original method for adsorption of the sera to detect anti-CAGTA and evaluate one of them for use in the diagnosis of invasive candidiasis.

The 154 serum specimens used for the comparison of the three methods of adsorption were submitted to the Department of Microbiology and Immunology, School of Medicine and Odontology, Basque Country University, for detection of anti-CAGTA. The value of method 2 in the diagnosis of invasive candidiasis was determined by using sera submitted to the Mycological Reference Laboratory, Central Public Health Laboratory, Colindale, London, United Kingdom, for serological testing. According to the clinical and microbiological diagnoses, patients and sera were divided into two groups. Group 1 comprised 118 serum specimens from 26 patients with invasive candidiasis distributed as follows: 70 serum specimens from 12 patients with septicemia, 22 serum specimens from 5 patients with endophthalmitis, 17 serum specimens from 5 patients with liver infection, 5 serum specimens from 2 patients with endocarditis, 2 serum specimens from a patient with kidney abscess, and 2 serum specimens from a patient with bone infection. Group 2 comprised 136 serum specimens from 58 patients without invasive candidiasis distributed as follows: 19 serum specimens from 3 patients with transient candidemia, 66 serum specimens from 12 patients at risk (transplant recipients) and on oral antifungal drug prophylaxis (fluconazole or polyenes), 10 serum specimens from 10 patients with aspergillosis (invasive aspergillosis, aspergilloma, and other *Aspergillus* diseases), 10 serum specimens from 10 patients with cryptococcosis (meningitis, pneumonitis, and other cryptococcal infections), 18 serum specimens from 10 patients with other mycoses, including disseminated trichosporonosis, and mucormycosis, and 13 serum specimens from 13 blood donors.

C. albicans serotype A (NCPF 3153) was grown as germ tubes or blastospores in medium 199 (Sigma, St. Louis, Mo.) as described previously (5). Washed germ tubes were used in the preparation of immunofluorescence slides (5). Heat-killed blastospores of the same strain were used in the adsorption of the sera. The adsorbing suspension was prepared by heating the blastospores at 60°C for 2 h. After three washes in saline, the organisms were counted in a hemacytometer and suspended at 10^{10} cells per ml.

Three methods were used for adsorption of the sera. Method 1 has been described previously (6). Briefly, sera were adsorbed four times by mixing equal volumes (100 μl) of the adsorbing suspension and sera. After incubation in a rotatory mixer for 2 h at room temperature, the organisms

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TABLE 1. Distribution of anti-CAGTA titers in the sera studied

Titer	No. of patients							
	Group 1 patients			Group 2 patients				
	Candida septicemia	Other invasive candidiases	Total	Transient candidemia	Neutropenia	Other mycoses	Blood donors	Total
Negative	23	9	32	16	50	30	13	109
1:20–1:80	22	1	23	3	3	3	0	9
1:160–1:640	21	13	34	0	10	5	0	15
1:1,280–1:5,120	4	15	19	0	3	0	0	3
1:10,240–1:20,480	0	10	10	0	0	0	0	0
Total	70	48	118	19	66	38	13	136

were removed by centrifugation. Adsorptions were repeated with supernatants twice at room temperature for 2 h and once more at 4°C for 18 h. Supernatants from the last adsorption (final dilution, 1:16) were adjusted to 1:20 and were used for antibody detection. In method 2, 25 μl of each serum specimen was adsorbed once with 375 μl of the adsorbing suspension. Adsorption was performed with rotation by placing the tubes on a turntable which inverted the tubes repeatedly for 2 h at room temperature. Supernatants (final dilution, 1:16) were adjusted to 1:20 and were used for antibody detection. In method 3, 24 μl of each serum specimen was diluted 1:4 in phosphate-buffered saline and was then adsorbed with 96 μl of the adsorbing suspension. Adsorption was performed as described above for method 2, and 50 μl of each supernatant was adsorbed again with the same volume of the adsorbing suspension. Supernatants from the last adsorption (final dilution, 1:16) were adjusted to 1:20 and were processed for antibody detection.

Anti-*C. albicans* antibodies were detected in adsorbed sera by an immunofluorescence antibody assay as described previously (6). Briefly, 10 μl of the sera or their serial dilutions were applied to the wells of Teflon-coated immunofluorescence slides to which *C. albicans* blastospores and germ tubes had been fixed. After incubation at 37°C for 30 min, slides were washed and incubated with fluorescein-conjugated goat anti-human immunoglobulin (Wellcome Reagents Ltd., Beckenham, England) at 37°C for another 30 min. The slides were washed again and examined with a Leitz Dialux microscope equipped for epifluorescence. Sensitivity, specificity, efficiency, and positive and negative predictive values were calculated as described by Kozinn et al., (3). StatView 512+ statistical software for the Apple Macintosh was used for computing the statistics.

The original method of adsorption and two modifications that simplify and shorten serum adsorption were initially compared with 154 serum specimens which included both anti-CAGTA-positive and -negative sera. Adsorption of the sera by methods 2 and 3 resulted in titers very similar to those obtained by method 1. In fact, 72.1% of serum specimens tested by method 2 and 69.5% of serum specimens tested by method 3 gave the same titer as that obtained by method 1. Titer discrepancies of one dilution with respect to titers detected by method 1 were observed in 22.7% of serum specimens tested by method 2 and 24.7% of serum specimens tested by method 3. Overall, only 5.2% of serum specimens tested by method 2 and 5.8% of serum specimens tested by method 3 presented discrepancies of one dilution or more with respect to the titer observed by method 1. Both modifications of the process of adsorption of the sera tended to increase the sensitivity of anti-*C. albicans* antibody detection, since discrepancies were more likely to produce titers higher than those detected by method 1. The similarity in the results obtained among the methods and the economy of time in method 2 (one adsorption versus two and four adsorptions by methods 3 and 1, respectively) led to selection of method 2 as the more convenient modification for the adsorption of the sera.

When a test based on method 2 was evaluated with sera from patients with invasive candidiasis, results were not very different from those obtained in previous studies by the original adsorption method when the criterion for considering the test positive was the presence of anti-CAGTA and not the titer (7). Anti-CAGTA were detected in 23 of 26 group 1 patients (88.5%). A titer of 1:20 or greater was observed in 86 of 118 serum specimens from group 1 patients. Anti-CAGTA were also present in 12 of 58 group 2 patients (27 of 136 serum specimens were positive). Statistically significant higher titers of anti-CAGTA were observed in group 1 patients than in group 2 patients ($P \leq 0.001$). Patients with invasive candidiasis other than *Candida* septicemia showed the highest titers (>1:20,480), as shown in Table 1. Three patients with invasive candidiasis did not show anti-CAGTA in their sera. One of them was a 2-year-old boy with hepatic candidiasis (caused by *C. albicans*) and who was treated with liposomal amphotericin B and flucytosine. The second of these patients presented with endophthalmitis; all of his sera were negative. The third patient was a child with Leiner disease (*Erythroderma desquamativum*) and a *Candida parapsilosis* septicemia treated with amphotericin B and flucytosine.

Titers of ≥1:160 were seen in 7 of 58 group 2 patients. Four of them were neutropenic bone marrow transplant recipients on prophylaxis with oral antifungal drugs. Diagnosis of disease in this group of patients is difficult. They are at risk of fungal infection, but the false-positive antibody detection results could be related to the presence of an invasive candidiasis obscured by the prophylactic treatment. The other three patients with anti-CAGTA of ≥1:160 suffered from invasive non-*Candida* mycoses. Two of them had cryptococcal meningitis, and the other one had disseminated trichosporonosis. Patients with cryptococcosis had titers of 1:160, and the patient with *Trichosporon* infection showed titers ranging from 0 to 1:640. The presence of a mixed infection with *Candida* spp. could not be discounted. Another possible explanation might be the presence of antigenic cross-reactivity, but the likelihood for this possibility must be low, since other patients with these infections did not have anti-CAGTA.

The ability of the test to discriminate between group 1 and group 2 patients was assessed by considering different titers

of anti-CAGTA (namely, ≥1:20, ≥1:40, ≥1:80, and ≥1:160). Sensitivity ranged from 88.5% (for a titer of ≥1:20) to 84.6% (for a titer ≥1:160). The best specificity of the test (87.9%) was obtained when a titer of ≥1:160 was considered positive. The test gave the best discriminatory results when this titer was used as the cutoff (sensitivity, 84.6%; specificity, 87.9%; positive predictive value, 75.9%; negative predictive value, 92.7%; efficiency, 86.9%). The decrease in specificity and positive predictive value observed by method 2 compared with previously published results obtained by method 1 (7) may be related to the higher sensitivity of the new adsorption method. Indeed, discrepancies observed in sera adsorbed by methods 1 and 2 were more likely to increase the anti-CAGTA titers. An increase in specificity can be obtained by establishing a titer of ≥1:160 as the cutoff. By using this titer, the specificity, efficiency, and positive predictive values increased, with only a low decrease in sensitivity.

We conclude that the modified test is simpler to perform without its value being impaired. In practice, sera could be screened at dilutions of ≥1:20 for general discrimination and ≥1:160 for groups of patients in whom the diagnosis is problematic. Such an approach could be useful when large numbers of patients are being screened. Usually, however, titrations of sera would be preferable, since they provide a clearer indication of the patient's antibody profile during management.

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