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Syscan3, a Kit for Detection of Anti-*Candida* Antibodies for Diagnosis of Invasive Candidiasis

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Sera from 76 immunocompetent and 293 immunocompromised subjects were assayed for anti-*Candida* antibodies. The sensitivity, specificity, positive predictive value, and negative predictive value for invasive candidiasis were 74%, 75%, 62%, and 84% in the immunocompetent group and 15%, 60%, 1.7%, and 93% in the immunocompromised group, respectively. Syscan3 has high negative predictive value.

Despite a recent trend of emerging mold infections among immunocompromised patients, candidemia is still the fourth most common nosocomial bloodstream infection in the United States (9). Attributable mortality rates are usually between 38 and 49% (7). It is well known that *Candida* is cultured from blood in only 50 to 70% of patients with disseminated candidiasis (4).

Diagnostic markers for invasive candidiasis have long been sought. Detection systems for metabolites (e.g., fungal D-arabinitol, (5, 10), fungal DNA by PCR (1, 8, 11), and fungal cell wall components (e.g., β-D-glucan) (13, 14) have all been investigated, but none has yet achieved broad validation. Assays for fungal antigens (or antibodies to same) have also been studied, but generally with little success (16).

The anti-*Candida* antibody approaches studied to date have included the Virotech and Biomerica kits (both use polyclonal antibodies) and the monoclonal antibody-based Platelia Candida kit. These kits have shown sensitivities ranging from 50 to 90% and specificities of ~15 to 65% (15). As its use of a mixture of target antigens offered the potential for increased diagnostic power, we evaluated a new anti-*Candida* antibody detection enzyme-linked immunosorbent assay-based kit (Syscan3; Rockeby Biomed Ltd.) as a potential adjunct for the diagnosis of invasive candidiasis.

Patient samples. Two frozen (−80°C) deidentified and anonymized serum collections were used. Collection A consisted of 76 subjects: 27 hospitalized patients with proven invasive candidiasis (26 with candidemia and 1 with candidal peritonitis), 6 hospitalized patients with noncandidal fungal infections (4 with cryptococcosis and 2 with invasive mold infections), and 43 healthy control subjects. The *Candida* species distribution was as follows: *C. albicans*, 16/27; *C. glabrata*, 4/27; *C. tropicalis*, 3/27; coinfection with *C. albicans* and *C. glabrata*, 2/27; and *C. krusei* and *C. parapsilosis*, 1 each. Sera from patients with invasive candidiasis were drawn within 96 h of the positive culture. Collection B consisted of sera collected twice weekly from 293 patients with acute myelogenous leukemia and myelodysplastic syndrome undergoing induction chemotherapy and receiving antifungal prophylaxis with either itraconazole or caspofungin. Thirteen of these patients were found to have proven or probable invasive candidiasis. The species distribution was as follows: *C. glabrata*, 7/13; *C. albicans*, 2/13; *C. krusei*, 2/13; and *C. parapsilosis* and *C. tropicalis*, 1 each. A single sample was selected for testing from each patient. For patients with invasive candidiasis, a sample was selected within 96 h of the diagnosis or the positive culture. For uninfected patients; the first available sample was utilized. Invasive candidiasis was defined according to the EORTC/MSG criteria (3). This study was approved by the UTHSCH Institutional Review Board.

Testing method. Syscan3 testing kits were provided by Rockeby Biomed Ltd. (Western Australia, Australia). Samples from each collection were mixed with a phosphate-buffered saline diluent (10 μl of serum mixed into 990 μl of serum diluent). One hundred microliters of the respective dilutions was deposited into the wells of a 96-well microdilution tray in which the wells had been coated with a proprietary mixture of purified intracytoplasmic *Candida* antigens, with enolase as the predominant antigen. Samples were incubated for 45 min at room temperature, washed, and incubated with horseradish peroxidase-conjugated antihuman antibodies for 45 min. After washing the wells with buffer, a peroxidase solution and acid stop solution were added. Sample absorbance was read using a dual-wavelength spectrophotometer at 450 nm with a reference of 650 nm. The kit also included positive, negative, and cutoff controls. Controls and sera were tested in duplicate. The reading for each test sample was determined in arbitrary units as (sample absorbance × 10)/(mean absorbance of cutoff control sample). The negative and positive controls had to fall within predetermined quality control ranges to accept the results as valid. A cutoff of ≥15 U was selected based on a preliminary study by the kit developers and by determining receiver operating characteristic curves with collection A (data

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TABLE 1. Diagnostic performance of SysCan3 with immunocompetent and immunocompromised hosts

Population (*n*)	No. of:				Sensitivity (%)	Specificity (%)	Predictive value (%)		Diagnostic accuracy (%)
	True positives	True negatives	False positives	False negatives			Positive	Negative	
Immunocompetent (76)	20	37	12	7	74	75	62	84	75
Immunocompromised (293)	2	170	110	11	15	60	1.7	93	58

not shown). Means of units between groups were compared by *t* test (SPSS 12.0.1; SPPS, Inc.), and diagnostic test performance was evaluated using standard formulas.

Results. For collection A, the mean numbers of units ± standard deviation in patients versus controls were 20.78 ± 6.81 U and 11.24 ± 5.94 U ($P < 0.0001$). For collection B, the mean numbers of units ± standard deviation in patients versus controls were 10.98 ± 6.58 U and 13.85 ± 6.98 U ($P = 0.15$). Using the cutoff of ≥15 U, for collection A, 20 of the 27 patients with invasive candidiasis were detected by Syscan3, while only 2 of 13 patients in collection B were identified. All of the patients with non-*Candida* fungal infections had negative results. As shown in Table 1, the sensitivity, specificity, positive predictive value, and negative predictive value for collection A were 74%, 75%, 62%, and 84%, respectively, while for collection B they were 15%, 60%, 1.7%, and 93%. Decreasing or increasing the cutoff for collection B did not improve the performance of the test.

Our evaluation of Syscan3 in both collections A and B yielded relatively high negative predictive values when compared to previously available commercial kits (15), thus offering a good possibility of "ruling out" the disease. The significance of the negative and positive predictive values should be approached with caution in collection A since these values rely on the prevalence of disease in the study population, which in the case of this collection, was artificially constructed (12). In the immunocompromised hematological patients evaluated in collection B, sensitivity and positive predictive values were low. Of note, the mean test results for the cases in collection B were similar to the values of controls in both collections. This might be due to reduced antibody production in the immunocompromised population (6) or the use of antifungal prophylaxis, thus preemptively treating infections in this subject group. Although the negative predictive value appears to be fairly good for collection B, the negative likelihood ratio is 0.38; thus, a negative test may result in only a small decrease in the probability that a patient in this population has an invasive *Candida* infection. Nevertheless, a statistical artifact related to the low prevalence of the studied disease cannot be excluded (12).

In summary, the Syscan3 kit offers reasonable negative predictive value. A prospective, multicenter evaluation of Syscan3 kit is needed to further evaluate its clinical usefulness in immunocompetent and other immunocompromised patients (e.g., human immunodeficiency virus-infected and organ transplant patients), as well as settings (such as intensive care units) with a high prevalence of invasive candidiasis. The effects of *Candida* colonization and superficial *Candida* infections remain to be studied. Also, repeat testing with multiple samples during the course of infection may help in more accurately diagnosing invasive candidiasis, as has been previously noted with other serological tests (2, 6, 17).

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REFERENCES

1. **Ahmad, S., A. S. Mustafa, Z. Khan, A. I. Al-Rifaiy, and Z. U. Khan.** 2004. PCR-enzyme immunoassay of rDNA in the diagnosis of candidemia and comparison with amplicon detection by agarose gel electrophoresis. Int. J. Med. Microbiol. **294:**45–51.
2. **Alexander, B. D.** 2002. Diagnosis of fungal infection: new technologies for the mycology laboratory. Transplant. Infect. Dis. **4**(Suppl. 3)**:**32–37.
3. **Ascioglu, S., J. H. Rex, B. de Pauw, J. E. Bennett, J. Bille, F. Crokaert, D. W. Denning, J. P. Donnelly, J. E. Edwards, Z. Erjavec, D. Fiere, O. Lortholary, J. Maertens, J. F. Meis, T. F. Patterson, J. Ritter, D. Selleslag, P. M. Shah, D. A. Stevens, and T. J. Walsh.** 2002. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin. Infect. Dis. **34:**7–14.
4. **Berenguer, J., M. Buck, F. Witebsky, F. Stock, P. A. Pizzo, and T. J. Walsh.** 1993. Lysis-centrifugation blood cultures in the detection of tissue-proven invasive candidiasis. Disseminated versus single-organ infection. Diagn. Microbiol. Infect. Dis. **17:**103–109.
5. **Christensson, B., G. Sigmundsdottir, and L. Larsson.** 1999. D-Arabinitol—a marker for invasive candidiasis. Med. Mycol. **37:**391–396.
6. **Denning, D. W., E. G. Evans, C. C. Kibbler, M. D. Richardson, M. M. Roberts, T. R. Rogers, D. W. Warnock, R. E. Warren et al.** 1997. Guidelines for the investigation of invasive fungal infections in haematological malignancy and solid organ transplantation. Eur. J. Clin. Microbiol. Infect. Dis. **16:**424–436.
7. **Gudlaugsson, O., S. Gillespie, K. Lee, J. Vande Berg, J. Hu, S. Messer, L. Herwaldt, M. Pfaller, and D. Diekema.** 2003. Attributable mortality of nosocomial candidemia, revisited. Clin. Infect. Dis. **37:**1172–1177.
8. **Iwen, P. C., A. G. Freifeld, T. A. Bruening, and S. H. Hinrichs.** 2004. Use of a panfungal PCR assay for detection of fungal pathogens in a commercial blood culture system. J. Clin. Microbiol. **42:**2292–2293.
9. **Jarvis, W. R.** 1995. Epidemiology of nosocomial fungal infections, with emphasis on Candida species. Clin. Infect. Dis. **20:**1526–1530.
10. **Kiehn, T. E., E. M. Bernard, J. W. Gold, and D. Armstrong.** 1979. Candidiasis: detection by gas-liquid chromatography of D-arabinitol, a fungal metabolite, in human serum. Science **206:**577–580.
11. **Maaroufi, Y., C. Heymans, J.-M. De Bruyne, V. Duchateau, H. Rodriguez-Villalobos, M. Aoun, and F. Crokaert.** 2003. Rapid detection of *Candida albicans* in clinical blood samples by using a TaqMan-based PCR assay. J. Clin. Microbiol. **41:**3293–3298.
12. **Moyer, V. A., and K. A. Kennedy.** 2003. Understanding and using diagnostic tests. Clin. Perinatol. **30:**189–204.
13. **Obayashi, T., M. Yoshida, T. Mori, H. Goto, A. Yasuoka, H. Iwasaki, H. Teshima, S. Kohno, A. Horiuchi, A. Ito et al.** 1995. Plasma (1→3)-beta-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. Lancet **345:**17–20.
14. **Odabasi, Z., G. Mattiuzzi, E. Estey, H. Kantarjian, F. Saeki, R. J. Ridge, P. A. Ketchum, M. A. Finkelman, J. H. Rex, and L. Ostrosky-Zeichner.** 2004. Beta-D-glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. Clin. Infect. Dis. **39:**199–205.
15. **Persat, F., R. Topenot, M. A. Piens, A. Thiebaut, E. Dannaoui, and S. Picot.** 2002. Evaluation of different commercial ELISA methods for the serodiagnosis of systemic candidosis. Mycoses **45:**455–460.
16. **Phillips, P., A. Dowd, P. Jewesson, G. Radigan, M. G. Tweeddale, A. Clarke, I. Geere, and M. Kelly.** 1990. Nonvalue of antigen detection immunoassays for diagnosis of candidemia. J. Clin. Microbiol. **28:**2320–2326.
17. **Walsh, T. J., J. W. Hathorn, J. D. Sobel, W. G. Merz, V. Sanchez, S. M. Maret, H. R. Buckley, M. A. Pfaller, R. Schaufele, C. Sliva et al.** 1991. Detection of circulating candida enolase by immunoassay in patients with cancer and invasive candidiasis. N. Engl. J. Med. **324:**1026–1031.