Evaluation of a newly developed down-flow immunoassay for detection of serum mannan antigens in patients with candidaemia

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A down-flow immunoassay has been developed to detect serum mannan antigens, and the test was recently marketed as the Unimedi *Candida* monotest. Using 251 serum samples from 105 patients with candidaemia, a comparison of the Unimedi *Candida* monotest with the Cand-Tec latex agglutination test and 2 microplate enzyme immunoassay tests (Platelia *Candida* Ag test and Unimedi *Candida*) was conducted. One hundred and seventy-five febrile patients without clinical and microbiological evidence of fungal infections and pneumocytosis were examined as controls. The Cand-Tec test had a sensitivity of 38 % and a specificity of 82 %. The sensitivity and specificity of the Platelia *Candida* Ag test, the Unimedi *Candida* and the Unimedi *Candida* monotest were 53 and 92 %, 69 and 89 % and 82 and 96 %, respectively. The sensitivity of the Unimedi *Candida* monotest was significantly ($P < 0.01$) higher than that of the Platelia *Candida* Ag test for diagnosing candidaemia caused by *Candida parapsilosis*. The β -D-glucan assay had a high sensitivity of 95 %, with a specificity of 84 %. Of 74 patients with candidaemia whose sera were available before or on positive blood culture sampling, 29 (39 %), 38 (51 %) and 48 (65 %) patients had antigenemia detected using the Platelia *Candida* Ag test, the Unimedi *Candida* and the Unimedi *Candida* monotest, respectively. The Unimedi *Candida* monotest seems to be a promising tool for the early diagnosis of invasive candidiasis, because the test was sensitive, simple, rapid (approx. 1 h) and cost-effective.

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INTRODUCTION

Yeasts of the genus *Candida* are a major cause of invasive infections in hospitalized patients receiving intensive medical care, particularly in neutropenic cancer patients and patients with indwelling intravenous catheters (Hajjeh *et al.*, 2004). The accurate and timely diagnosis of invasive candidiasis is difficult due to nonspecific clinical signs and the natural commensal status of these opportunistic pathogens. In order to overcome these difficulties, non-culture methods have been developed. With these methods, highly sensitive and specific detection of fungal DNA from clinical materials has been demonstrated (Maaroufi *et al.*, 2003; Ahmad *et al.*, 2004). However, technical problems such as the risk of contamination and the inability to distinguish colonized cases from infected patients may need to be solved. Moreover, methods for DNA extraction and DNA detection should be both standardized and simplified for the introduction of a molecular diagnostic method to routine clinical laboratories.

The following findings or tests are used for the diagnosis of invasive candidiasis in clinical laboratories: isolation of *Candida* species from normally sterile sites, histopathological evidence of *Candida* invasion, endophthalmitis, hepatosplenic lesions detected by imaging techniques, detection of anti-*Candida albicans* antibodies (Sendid *et al.*, 1999), and detection of *Candida* components such as mannan (Yeo & Wong, 2002), D-arabinitol (Walsh *et al.*, 1995) and (1 $\rightarrow$ 3)- β -D-glucan (BG) (Obayashi *et al.*, 1995). Unfortunately, all of these assays have limitations reflected by their sensitivity and/or specificity. Therefore, a combination of two or more assays is necessary for accurate diagnosis of invasive candidiasis (Sendid *et al.*, 2002; Yeo & Wong, 2002).

Detection of serum mannan has been developed using a microplate enzyme immunoassay, and an assay kit is commercially available as the Platelia *Candida* Ag test (Bio-Rad). However, the sensitivity of this test has been reported to be 40 % for patients with invasive candidiasis caused by *C. albicans* (Sendid *et al.*, 1999). Recently a new enzyme immunoassay test for the detection of mannan antigens, the Unimedi *Candida* monotest (Unitika), has become available

Abbreviation: BG, (1 $\rightarrow$ 3)- β -D-glucan.

commercially. The objective of this study was to clarify the usefulness of the new assay for diagnosing invasive candidiasis by comparison with commercial kits for serum antigens and plasma BG.

METHODS

Preparation of antigen extracts. A total of 48 blood culture *Candida* isolates (5 isolates of *C. albicans*, 2 of *Candida dubliniensis*, 13 of *Candida glabrata*, 5 of *Candida guilliermondii*, 2 of *Candida krusei*, 15 of *Candida parapsilosis* and 6 of *Candida tropicalis*) and 5 *Candida* control strains were used to study the cross-reactivity of 2 enzyme immunoassays, the Platelia *Candida* Ag test and the Unimedi *Candida* monotest. The *Candida* control strains were obtained from the NITE Biological and Technological Center, Kisarazu, Japan (NBRC), and included *Candida kefyr* (NBRC942, NBRC1065), *Candida norvegensis* (NBRC1020) and *Candida rugosa* (NBRC1152, NBRC2901). Each strain grown on CHROMagar *Candida* (Hardy Laboratories) was inoculated into RPMI 1640 medium (Dainippon Pharmaceutical) and incubated at 35 °C for 2–3 days. The culture medium was boiled in a water bath for 7 min. After centrifugation, the supernatant was diluted with the same medium at 1:1000, and the diluted solutions were tested for mannan antigens.

Patients. Candidaemic patients admitted to Kanazawa University Hospital during the period January 1995 to July 2005 were included in the present study, provided that at least two serum samples taken during the fever episode were available. Candidaemia was diagnosed on the basis of at least one blood culture positive for *Candida*

species, together with a temperature of more than 38 °C, which was refractory to antibiotics for more than 2 days. Antifungal drugs were given intravenously to all candidaemic patients. A total of 105 patients (37 females and 68 males, mean age 52·8 years) were selected, and these included 49 (47%) patients infected with *C. albicans*, 11 with *C. glabrata*, 11 with *C. guilliermondii*, 20 with *C. parapsilosis*, 9 with *C. tropicalis* and 5 with other *Candida* species. When two or more episodes of candidaemia were observed in one patient, the first episode was included. Blood cultures and identification of yeast isolates were performed as described previously (Fujita *et al.*, 2001). The clinical and mycological findings are summarized in Table 1. Of the 105 candidaemic patients, 69 (66%) had a solid tumour, 6 (6%) had cardiovascular disease, 5 (5%) had leukaemia, 3 (3%) had malignant lymphoma and 22 (20%) had other disorders. The intravenous catheter tip cultures grew *Candida* belonging to the same species as that of blood isolate in 49 (47%) candidaemic patients. Control febrile patients who had no clinical evidence of fungal findings and were treated successfully without systemic antifungal drugs included 105 bacteraemic patients and 70 non-bacteraemic patients. None of the control patients also showed clinical evidence of pneumocytosis. The 105 blood cultures included 37 (35%) Gram-negative bacteria (7 were identified as *Escherichia coli*, 9 as *Klebsiella* spp., 4 as *Enterobacter* spp., 9 as *Pseudomonas aeruginosa* and 8 as other Gram-negative bacteria). A total of 68 (65%) of the 105 blood cultures contained Gram-positive bacteria (19 were identified as *Staphylococcus aureus*, 18 as *Staphylococcus epidermidis*, 12 as coagulase-negative staphylococci, 11 as *Enterococcus* spp. and 8 as other Gram-positive bacteria).

Detection of *Candida* antigens. A total of 251 serum samples obtained during the 7 days before and 4 days after the first positive

Table 1. Clinical characteristics of candidaemic patients by *Candida* species

Characteristic	No. of patients by species					
	<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. guilliermondii</i>	<i>C. parapsilosis</i>	<i>C. tropicalis</i>	Other
Total no. of patients	49	11	11	20	9	5
Total no. of serum samples	118	25	27	50	20	11
Underlying disease:						
Leukaemia	2	—	1	—	—	2
Malignant lymphoma	1	—	1	1	—	—
Solid tumour	29	8	7	14	8	3
Cardiovascular disease	5	—	—	1	—	—
Other	12	3	2	4	1	—
Neutropenia (<1000 leukocytes mm ⁻³)	2	—	—	—	—	2
<i>Candida</i> recovered from:						
Intravenous catheter	21	5	5	13	3	2
Urine	5	1	—	1	1	1
Sputum	2	1	1	—	1	—
Bile	1	—	—	—	—	—
Exudate	2	1	1	—	—	—
Antifungal drugs before candidaemia:						
Amphotericin B (oral)	3	—	2	1	—	2
Itraconazole	—	1	—	—	—	—
Fluconazole	—	1	—	—	—	—
Treatment of candidaemia:						
Amphotericin B	1	—	1	1	—	1
Fluconazole	41	8	10	17	8	3
Micafungin	7	3	—	2	1	1
Died within 30 days after candidaemia	20	1	1	3	5	2

blood culture sampling were examined retrospectively for *Candida* antigens. Of the 251 serum samples from 105 patients with candidaemia, 110 serum samples (1.0 sample per patient) were tested using the Cand-Tec assay (Ramco Laboratories), and 251 samples (2.4 samples per patient) using the Platelia *Candida* Ag test, the Unimedi *Candida* assay (Unitica) and the Unimedi *Candida* monotest.

For the Cand-Tec assay, sera were diluted 1:4 with the diluent supplied in the kit. Twenty microlitres of the diluted specimen was mixed with the same volume of latex particles coated with anti-*Candida* antibody. The mixture was rotated at 140 r.p.m. for 10 min then checked immediately for agglutination.

The Platelia *Candida* Ag test, which employs mAb that recognizes the alpha-linked mannopentose of *C. albicans* mannan, was performed as described by Sendid *et al.* (1999). Each experiment included a calibration curve, which was made with a pool of normal human serum supplemented with known mannan antigen concentrations (0.25, 0.5, 1.0 and 2.0 ng ml⁻¹). Although the test detected as little as 0.25 ng serum mannan ml⁻¹, mannan concentrations greater than 0.5 ng ml⁻¹ were considered a positive result, according to the manufacturer's recommendations.

The Unimedi *Candida* test involves affinity-purified antibodies. Antisera were prepared by immunizing Japanese white rabbits with intravenous injections of 0.5 mg (1 mg ml⁻¹ in PBS) of formalin-killed and acetone-dried cells of *C. albicans* YD1124 twice a week. After eight inoculations, blood was collected, and sera were treated using a mannan-fixed formyl-cellulofine column. Mannan antigens were prepared by the method reported by Reiss *et al.* (1986). A total of 150 µl serum sample was mixed with 150 µl of treatment solution (20 mM NaH₂PO₄), boiled for 4 min, and then centrifuged at 10 000 r.p.m. for 10 min. One hundred microlitres of supernatant was added to antibody-coated microtitre plate wells. After incubation at room temperature for 2 h, the plates were washed thoroughly and 100 µl alkaline phosphatase-conjugated antibodies was added to the wells. After further incubation at room temperature for 1 h, the plates were washed. The reaction was revealed by incubation with 100 µl substrate solution (50 mM Tris(hydroxymethyl)aminomethane, 2.2 mM *p*-iodonitrotetrazolium violet, 0.3 mM NADPH) for 20 min at room temperature. After addition of stopping solution (1 M phosphate) to the wells, the A₄₉₀ was read on a microplate reader. In the preliminary study, the test detected as little as 0.25 ng mannan ml⁻¹ when the antigen was seeded into pooled human serum.

The Unimedi *Candida* monotest (a down-flow immunoassay) was used according to the manufacturer's instructions. Briefly, aliquots of 200 µl serum sample were denatured with 200 µl treatment solution, and the mixture was boiled for 4 min and then centrifuged at 10 000 r.p.m. for 10 min. A total of 200 µl supernatant were mixed in microcentrifuge tubes with 100 µl alkaline phosphatase-conjugated antibodies, which were the same antibodies as used in the Unimedi *Candida* kit. Following incubation for 30 min at room temperature, 250 µl of the mixture was applied to the loading window of the cassettes. After drying, the cassettes were washed with 10 drops of wash solution, and then 4 drops of the substrate solution (0.5 mM 5-bromo-4-chloro-3-indolyl phosphate, 0.5 mM nitrotetrazolium blue) was added to the loading window. After 10 min of incubation at room temperature, four drops of stopping solution (0.1 M phosphate) were added, and the reactions (purple colour) appeared on the anti-mannan antibodies coated nitrocellulose membrane were classified visually into four categories according to the photographs of the standards; the intensity of reactions obtained with pooled serum samples spiked with mannan at concentrations of 0, 0.125, 0.25 and 0.5 ng ml⁻¹ were scored as - (negative), 1+, 2+ and 3+, respectively. The sensitivity of this method in heat-treated sera was 0.125 ng ml⁻¹.

BG assay. Of 105 patients with candidaemia, 76 patients (90 sera) were studied for plasma BG levels. The kinetic turbidimetric *Limulus* method (BG test; Wako) was used prospectively for quantification of plasma BG as described by Mori *et al.* (1997). Briefly, blood samples collected using a syringe, the inside wall of which was coated with a small amount of heparin, were centrifuged at 3000 r.p.m. for 40 s. Aliquots of 0.1 ml plasma samples were added to 0.9 ml pretreatment solution, and heated at 70 °C for 10 min. Then, 0.2 ml aliquots of the pretreated samples were added to the *Limulus* amoebocyte lysate reagent, and the turbidities of the resultant mixtures were measured using a Toxinometer MT-358 (Wako). The cut-off value was 11 pg BG ml⁻¹.

Statistical analysis. The chi-square test and Fisher's exact probability test were employed by use of computer software (Statview statistical software package; Abacus Concepts). Statistical significance was assured when the *P* value was less than 0.05.

RESULTS AND DISCUSSION

Both the Platelia *Candida* Ag test and the Unimedi *Candida* monotest revealed strongly positive reactions with the strains of *C. albicans*, *C. dubliniensis*, *C. rugosa* and *C. tropicalis*, compared to negative or weakly positive reactions with culture fluids of *C. kefyr* and *C. norvegensis*. Concentrations of antigens obtained from *C. guilliermondii*, *C. krusei* and *C. parapsilosis* differed between the two assays: the Unimedi *Candida* monotest showed stronger reactions than the Platelia *Candida* Ag test. Interestingly, 9 (60 %) of 15 *C. parapsilosis* strains and 2 of 5 *C. guilliermondii* strains showed negative reactions with the Platelia *Candida* Ag test, while none of these strains tested showed negative reactions with the Unimedi *Candida* monotest. Furthermore, the reactions obtained with each assay varied from weak to strong when culture fluids of *C. glabrata* strains were tested. Table 2 shows the antigen levels in 64 sera obtained from 27 patients who had two or more positive blood cultures and in culture fluids of the blood culture isolate. All patients infected with *Candida* strains that showed negative or weakly positive reactions with the Platelia *Candida* Ag test or the Unimedi *Candida* monotest did not develop mannanaemia. In addition, three patients infected with *C. glabrata* isolates that showed strong reactions (>2.0 ng ml⁻¹) with the Platelia *Candida* Ag test and four patients infected with *Candida* strains (three of *C. glabrata* and one of *C. parapsilosis*) that showed strong reactions (scored as 3+) using the Unimedi *Candida* monotest did not have mannanaemia, suggesting incomplete dissociation of mannan-serum complexes.

Table 3 shows the results of assays for serum *Candida* antigens and plasma BG by *Candida* species isolated from blood cultures. Taking a titre of 1:4 or more for a positive Cand-Tec assay result, 40 (38 %) of 105 candidaemic patients and 32 (18 %) of 175 control patients were positive for antigens (38 % sensitivity and 82 % specificity). Three mannan antigen assays showed nearly 70 % or more positive reactions in patients with *C. albicans* and *C. tropicalis* infection. Of 20 patients (50 sera) with candidaemia due to *C. parapsilosis*, 3 (8 sera) showed mannanaemia with the Platelia *Candida* Ag test, and 17 (38 sera) with the Unimedi *Candida* monotest

Table 2. Cross-reactivity of enzyme immunoassay for the detection of mannan antigens from patients' sera and from culture fluids of the blood culture isolate

No. of patient	Blood isolate	No. of positive blood cultures	Serum antigen levels detected by:		Antigen levels in culture fluids* detected by:	
			Platelia <i>Candida</i> Ag (ng ml ⁻¹)†	Unimedi <i>Candida</i> monotest	Platelia <i>Candida</i> Ag (ng ml ⁻¹)	Unimedi <i>Candida</i> monotest
1	<i>C. albicans</i>	4	>2·0, 1·3, >2·0	3+, 3+, 3+	>2·0	3+
2	<i>C. albicans</i>	2	>2·0, 1·61	2+, 2+	>2·0	3+
3	<i>C. albicans</i>	2	1·33, <0·25	1+, -, -	>2·0	3+
4	<i>C. dubliniensis</i>	3	0·88, 0·95	1+, 1+	>2·0	3+
5	<i>C. glabrata</i>	3	<0·25, <0·25	-, -	1·3	1+
6	<i>C. glabrata</i>	2	<0·25, <0·25	-, -	>2·0	3+
7	<i>C. glabrata</i>	3	<0·25, <0·25	2+, 2+	1·1	3+
8	<i>C. glabrata</i>	2	0·31, <0·25	1+, 1+	>2·0	3+
9	<i>C. glabrata</i>	2	<0·25, <0·25, <0·25	-, -, -	1·35	3+
10	<i>C. glabrata</i>	2	<0·25, <0·25	-, -	>2·0	3+
11	<i>C. guilliermondii</i>	2	0·33, 0·29	1+, 1+	0·42	3+
12	<i>C. guilliermondii</i>	2	<0·25, <0·25, <0·25	-, -, -, 1+	<0·25	3+
13	<i>C. guilliermondii</i>	2	0·41, 0·33, 0·42	1+, 3+, 3+	0·64	3+
14	<i>C. guilliermondii</i>	2	0·41, 0·39	2+, 2+	0·88	3+
15	<i>C. krusei</i>	2	<0·25, <0·25	1+, 1+	<0·25	3+
16	<i>C. krusei</i>	3	0·39, <0·25	1+, 1+	<0·25	3+
17	<i>C. norvegensis</i>	3	<0·25, <0·25, <0·25	-, -, -	<0·25	1+
18	<i>C. parapsilosis</i>	3	0·26, <0·25, <0·25	3+, 3+, 3+	0·61	3+
19	<i>C. parapsilosis</i>	3	<0·25, <0·25	-, -	<0·25	3+
20	<i>C. parapsilosis</i>	2	<0·25, <0·25	2+, 3+	<0·25	3+
21	<i>C. parapsilosis</i>	4	<0·25, <0·25	3+, 3+	<0·25	3+
22	<i>C. parapsilosis</i>	2	<0·25, <0·25, <0·25	3+, 3+, 3+	<0·25	3+
23	<i>C. parapsilosis</i>	2	<0·25, 0·33	3+, 2+	<0·25	3+
24	<i>C. parapsilosis</i>	2	0·29, 0·34	2+, 3+	0·48	3+
25	<i>C. rugosa</i>	3	0·61, 0·55, 0·91	3+, 3+, 3+	>2·0	3+
26	<i>C. tropicalis</i>	3	1·21, 1·11	1+, 3+	>2·0	3+
27	<i>C. tropicalis</i>	2	>2·0, >2·0	3+, 3+	>2·0	3+

*Culture fluids were diluted at 1:1000 with RPMI 1640 medium.

†Bold type indicates positive results.

($P < 0.01$). The Platelia *Candida* Ag test revealed positive results in only 3 of 11 patients (3 of 27 sera) with *C. guilliermondii* infection, whereas the Unimedi *Candida* showed positive reactions in 9 patients (15 sera), and the Unimedi *Candida* monotest in 10 patients (20 sera). Two patients (three sera) with *C. krusei* infection showed mannanaemia on examination of the sera with the Unimedi *Candida* and Unimedi *Candida* monotest, but not with the Platelia *Candida* Ag test. Of 11 patients with *C. glabrata* infections, 4 patients (6 sera), 3 patients (5 sera) and 6 patients (11 sera) had mannanaemia detected by the Platelia *Candida* Ag test, the Unimedi *Candida* and the Unimedi *Candida* monotest, respectively. A total of 7 (4%) of 175 control patients showed antigenemia as determined with the Unimedi *Candida* monotest, as compared to 19 (11%) with the Unimedi *Candida* and 17 (7%) with the Platelia *Candida* Ag test. The sensitivity and specificity of the Platelia *Candida* Ag test, the Unimedi *Candida* and the Unimedi *Candida* monotest were 53% (45% per sera) and 92%, 69% (56%

per sera) and 89%, and 82% (73% per sera) and 96%, respectively.

Positive BG values (> 11 pg ml⁻¹) determined using the BG test (Wako) were observed in 72 (95%) of 76 patients (90 sera) with candidaemia: 5 with slightly elevated values ($11 - < 20$ pg ml⁻¹), 10 with moderately elevated values ($20 - < 30$ pg ml⁻¹) and 57 with highly elevated values (≥ 30 pg ml⁻¹). A total of 28 (16%) of 175 control patients (31 of 181 sera) showed elevated BG levels, ranging from slightly to highly elevated. The BG assay had a high sensitivity of 95%, with a specificity of 84%.

Of 74 patients with candidaemia whose sera were available before or on positive blood culture sampling, 29 (39%), 38 (51%) and 48 (65%) patients showed mannanaemia with the Platelia *Candida* Ag test, the Unimedi *Candida* and the Unimedi *Candida* monotest, respectively. A total of 17 (81%) of 21 patients had elevated BG levels before or on positive blood culture sampling.

Table 3. Results of serum *Candida* antigens and BG assays for patients with and without candidaemia

Group (no. of patients)	No. of antigen positive patients/no. of patients tested (no. of antigen positive sera/no. of sera tested) by:				No. of patients with positive BG values/no. of patients tested (no. of sera positive for BG/no. of sera tested)
	Cand-Tec	Platelia <i>Candida</i> Ag test	Unimedi <i>Candida</i>	Unimedi <i>Candida</i> monotest	
Candidaemia					
<i>C. albicans</i> (49)	24/49 (25/52)	38/49 (80/118)	37/49 (77/118)	40/49 (88/118)	31/31 (38/38)
<i>C. dubliniensis</i> (1)	1/1 (1/1)	1/1 (2/2)	1/1 (1/2)	1/1 (2/2)	0/1 (0/1)
<i>C. glabrata</i> (11)	3/11 (3/11)	4/11 (6/25)	3/11 (5/25)	6/11 (11/25)	9/9 (10/11)
<i>C. guilliermondii</i> (11)	2/11 (2/11)	3/11 (3/27)	9/11 (15/27)	10/11 (20/27)	7/7 (8/8)
<i>C. krusei</i> (2)	0/2 (0/2)	0/2 (0/3)	1/2 (1/3)	2/2 (3/3)	1/2 (2/2)
<i>C. norvegensis</i> (1)	0/1 (0/2)	0/1 (0/3)	0/1 (0/3)	0/1 (0/3)	1/1 (1/1)
<i>C. parapsilosis</i> (20)	6/20 (6/20)	3/20 (8/50)	13/20 (25/50)	17/20 (38/50)	16/17 (18/19)
<i>C. rugosa</i> (1)	0/1 (0/1)	1/1 (3/3)	1/1 (3/3)	1/1 (3/3)	1/1 (2/2)
<i>C. tropicalis</i> (9)	4/9 (4/10)	6/9 (11/20)	8/9 (15/20)	9/9 (16/20)	6/7 (6/8)
Subtotal	40/105 (42/110)	56/105 (112/251)	72/105 (141/251)	86/105 (182/251)	72/76 (85/90)
Sensitivity per patient	38 %	53 %	69 %	82 %	95 %
Sensitivity per serum	38 %	45 %	56 %	73 %	94 %
Febrile controls					
Bacteraemia (105)	20/105 (20/105)	11/105 (11/105)	15/105 (15/105)	5/105 (5/109)	18/105 (20/109)
Without bacteraemia (70)	12/70 (12/70)	3/70 (3/73)	4/70 (5/80)	2/70 (2/72)	10/70 (11/72)
Subtotal	32/175 (32/175)	14/175 (14/178)	19/175 (20/185)	7/175 (7/181)	28/175 (31/181)

The Cand-Tec was the first commercially available antigen test. As shown by other investigators (Yeo & Wong, 2002), the usefulness of this test seems to be limited because of the low sensitivity (38 % in our study) for diagnosing invasive candidiasis. Therefore, attempts to improve the immunological detection of mannan, involving the use of a more sensitive test format, and the use of monoclonal antibodies that react with defined epitopes, have been made (Yeo & Wong, 2002).

The Platelia *Candida* Ag test is based on the use of a monoclonal antibody, EB-CA1, which recognizes a mannopentose epitope of *C. albicans*. This epitope has also been found at high levels in *C. glabrata* and *C. tropicalis*, but has only been found to a lesser extent in *C. krusei*, *C. kefyr* and *C. parapsilosis* (Jacquinot *et al.*, 1998; Rimek *et al.*, 2003). With the exception of the results with *C. glabrata*, these previous reports support our results of antigen detection from serum samples of candidaemic patients and from culture fluids of *Candida* isolates. In contrast to the positive reactions of EB-CA1 with *C. guilliermondii* (Rimek *et al.*, 2003), we observed negative reactions with two strains and slightly or moderately positive reactions ($< 1.0 \text{ ng ml}^{-1}$) with the remaining three *C. guilliermondii* strains. As a result, only 3 (27 %) of 11 patients with candidaemia due to *C. guilliermondii* showed mannanaemia in the present study. According to the reported results (Sendid *et al.*, 2002), the sensitivity for the detection of mannanaemia with the Platelia *Candida* Ag test was 58–70 % for infections caused by *C. albicans*, *C. glabrata* and *C. tropicalis*, while it was 25–30 % for infections caused by *C. parapsilosis* and *C. krusei*. As for *C. glabrata*, the amounts of epitopes recognized by the antibodies used by

two assays (the Platelia *Candida* Ag test and the Unimedi *Candida* monotest) differed greatly from strain to strain, and may be less than for other clinically relevant *Candida* species, resulting in a low sensitivity for diagnosing *C. glabrata* infections. According to the reported results of mannan detection from 34 serum samples from 12 candidaemic patients (Yoshida *et al.*, 2002), the sensitivity of the Unimedi *Candida* has been higher than that achieved with the Platelia *Candida* Ag test (82.1 vs 40.0 %). This may have been caused by a difference in the reactivities of the respective kits against different species of *Candida*. Therefore, an increased proportion of patients with invasive candidiasis due to *C. parapsilosis* and *C. glabrata*, which are common non-*C. albicans* *Candida* species causing yeast blood stream infections (Hajjeh *et al.*, 2004; Pfaller *et al.*, 1998, 1999), may lower the sensitivity of the Platelia *Candida* Ag test.

The mannan assay employing affinity-purified polyclonal antibodies against *C. albicans* mannan has been marketed as the Unimedi *Candida* (a microplate enzyme immunoassay) since 2000. Recently, the Unimedi *Candida* monotest, which uses the same antibody as the Unimedi *Candida* assay, has become commercially available.

In the present study, the Unimedi *Candida* monotest was more sensitive and specific than the Unimedi *Candida* assay: the sensitivity improved from 69 to 82 %, and the specificity from 89 to 96 %. Moreover, the Unimedi *Candida* monotest has the advantage over previous mannan assays in that semi-quantitative results can be obtained within 1 h, and it requires no specialized instruments, such as microplate washer and microplate reader. In addition, the test is available for testing a single serum sample.

BG is a cell wall polysaccharide component specific for fungi except for *Mucor* spp., and to a lesser extent, cryptococci (Miyazaki *et al.*, 1995). Measurement of plasma BG is used widely in Japan as a test to screen a febrile patient for fungal infections, because quantitative results can be obtained within the same day of blood collection. Two methods are currently available for the sensitive detection of BG: a chromogenic assay and a kinetic turbidimetric assay. Elevated BG levels have been detected in >90 % of patients with candidaemia, with a specificity of >90 % (Mori *et al.*, 1997; Odabasi *et al.*, 2004). Although the sensitivity of the BG assay was high (95 %), the specificity was lower than those of the mannan antigen assays. In our series of patients, none of the control patients received haemodialysis and/or administration of antitumour BG products. Both of these are known to be factors resulting in false-positive BG measurements (Kanda *et al.*, 2001; Ishizuka *et al.*, 2004). There are two things that may contribute to false-positive results in the BG assay. First, administration of albumin products (Usami *et al.*, 2002) and/or immunoglobulin products (Ikemura *et al.*, 1989) before or during fever episodes may result in false-positive BG elevation. Second, BG may reach the circulation through damaged intestinal or respiratory mucosa and cause false-positive results in tests to detect BG. Because the BG assay had a high sensitivity of 95 % (94 % per serum), the assay seems to be most useful for excluding candidiasis.

Serological tests to detect *C. albicans* mannan and *C. albicans* antibodies have been positive in 73 % of patients with candidiasis at least 2 days before blood cultures became positive (Yera *et al.*, 2001). The present study showed that both mannan detection by the Unimedi *Candida* monotest and BG measurement by the kinetic turbidimetric assay were useful for the early diagnosis of invasive candidiasis. However, further studies of rapid and efficient methods for dissociating mannan from antibodies in serum, a major blocking factor (Lew *et al.*, 1982; Sendid *et al.*, 2002), are mandatory to improve the sensitivity of the assay.

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