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Cross-Reactivity of the PLATELIA *CANDIDA* Antigen Detection Enzyme Immunoassay with Fungal Antigen Extracts

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We studied the specificity of the PLATELIA *CANDIDA* Ag enzyme immunoassay by using 130 isolates of 63 clinically relevant fungal species. Antigen extracts of seven *Candida* spp. (*Candida albicans*, *C. dubliniensis*, *C. famata*, *C. glabrata*, *C. guilliermondii*, *C. lusitaniae*, and *C. tropicalis*) repeatedly yielded positive reactions (>0.5 ng/ml). *Geotrichum candidum* and *Fusarium verticillioides* were found to yield borderline-positive reactions (0.25 to 0.50 ng/ml). Antigen preparations from the other 54 fungal species, including yeasts, molds, dermatophytes, and dimorphic fungi, did not cross-react in the assay.

Invasive candidiasis is a major cause of mortality and morbidity among hospitalized patients. The diagnosis is difficult to establish because clinical symptoms are nonspecific. Fifty to 60% of cases of candidemia are caused by *Candida albicans*, followed by *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* (7, 8). Blood cultures, which are the "gold standard" for the diagnosis of disseminated candidiasis, lack sensitivity and usually take several days to show detectable growth of yeast cells (9). Therefore, nonculture methods like *Candida* DNA detection by PCR or tests for *Candida* antigen detection are being developed for the laboratory diagnosis.

Mannan is the main soluble immunodominant component of the outer cell wall layer of *Candida* species (6). The mannans are released from the cell walls of the yeasts during infection and circulate in the bloodstream (3, 4, 11). By using the monoclonal antibody EB-CA1 for the detection of α-linked oligomannoside antigens of *C. albicans*, a latex agglutination assay was introduced in 1991 (1); subsequently, a more sensitive, one-stage sandwich microplate enzyme immunoassay (i.e., the PLATELIA *CANDIDA* Ag EIA; Bio-Rad, Munich, Germany) was developed in 1999 (11). The detection limit of this assay is 0.25 ng of mannan per ml of serum, and the clinical specificity and sensitivity are 98 and 40%, respectively (11).

In the present study, we evaluated the PLATELIA *CANDIDA* Ag EIA for cross-reactivities with the cell wall mannan antigens of 63 clinically relevant fungal species, including yeasts, molds, dermatophytes, and dimorphic fungi.

Fungal strains. A total of 130 fungal strains of 63 clinically relevant species were tested, including 96 strains of yeasts, 27 strains of molds, 5 strains of dermatophytes, and 2 strains of dimorphic fungi (Table 1).

Preparation of antigen extracts. The fungal strains were grown on Sabouraud glucose (4%) agar plates. The blastoconidia of yeasts were harvested after 1 to 2 days of incubation at 28 or 37°C; the conidia and hyphal fragments of molds were harvested after 7 to 14 days of incubation at 28 or 37°C; the conidia and hyphal fragments of dermatophytes were harvested after 30 days of incubation at 28°C; and the blastoconidia (yeast phase) of dimorphic fungi were harvested after 10 days of incubation at 37°C.

For the preparation of antigen extracts, four to five colonies of yeasts and dimorphic fungi were suspended in 2 ml of sterile phosphate-buffered saline (PBS; pH 7.2). Molds and dermatophytes were harvested and suspended in sterile PBS by using wet swabs, yielding a suspension of both conidia and hyphal fragments, as confirmed by microscopic examination. The fungal suspensions were vortexed and adjusted to a density of McFarland 5.0. Aliquots of the McFarland 5.0 standard suspensions (500 μl) in PBS were transferred into 1.5-ml safe-lock Eppendorf tubes (Eppendorf, Hamburg, Germany), kept in a boiling water bath for 5 min, and subsequently centrifuged at 10,000 × *g* for 10 min. Thirty microliters of the supernatants was diluted into 300 μl of 6% bovine serum albumin solution (BSA; DiaMed AG, Cressier sur Morat, Switzerland).

Control of antigen extracts. Successful antigen extractions were confirmed by testing the extracts of *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. tamarii*, *A. terreus*, *Penicillium chrysogenum*, *P. marneffei*, and *Fusarium oxysporum* in the Platelia *Aspergillus* EIA (Bio-Rad), because these molds are known to cross-react with the antibody of this assay (5). All antigen extracts tested positive. The antigen extracts of the four *Cryptococcus neoformans* strains tested positive by the Pastorex *Crypto* Plus latex agglutination test (Bio-Rad).

Detection of mannan antigens by PLATELIA *CANDIDA* Ag EIA. The EIA (Bio-Rad) was performed according to the instructions of the manufacturer by using 300 μl of fungal antigen extracts diluted in BSA in place of patient's serum.

In each experiment, we included a total of seven control samples: (i) one negative control serum sample, (ii–v) a series of four standard positive control samples of known mannan antigen concentrations (0.25, 0.5, 1.0, and 2.0 ng/ml, respectively), (vi) one positive control serum sample with an antigen concentration between 1.0 and 2.0 ng/ml, and (vii) one control of BSA without fungal supplement.

Briefly, 300 μl of the experimental samples and the controls

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TABLE 1. Cross-reactivity of the PLATELIA *CANDIDA* Ag EIA with fungal antigen extracts

Fungal group	Fungal species	Strain designation(s)[a]	No. of strains with results equivalent to *Candida* antigen concn[b] of:		
			<0.25 (negative)	0.25–0.5 (borderline)	>0.5 (positive)
Yeasts	*Candida albicans*	ATCC 24433, ATCC 90028, ATCC 76615, ATCC 44374, B-765/96, B-833/96, EF-H500, EF-H1042, HD1278/99, HD1629/99, HD1642/99, HD3668/99, HD1419/00, HD1903/00, HD1936/00, HD3196/00, HD3311/00, HD3315/00, HD3322/00, HD1360/01, Gö-ThB	0	0	21
	Candida dubliniensis	EF-H1006, EF-H1007	0	0	2
	Candida famata	HD587/96, HD4645/00, HD1431/99, HD586/96, RV-4/97	0	1	4
	Candida glabrata	ATCC 90030, DSM11950, HD467/00, HD67/00, RV-6/97, RV-3/99	0	0	6
	Candida guilliermondii	ATCC 90877, HD545/95, HD2977/99, RV-2/96	0	0	4
	Candida lusitaniae	HD2501/98, HD2886/99, HD2937/99, RV-10/97	0	0	4
	Candida tropicalis	ATCC 28707, ATCC 90874, RV-7/98, RV-1/00	0	0	4
	Candida inconspicua	HD1538/00, RV-K10/96	2	0	0
	Candida kefyr	DSM11954, EF-H711, HD2060/99, RV-III/99	4	0	0
	Candida krusei	ATCC 90878, RV-D4/96, RV-2/98, RV-7/99	4	0	0
	Candida lipolytica	HD2632/98, RV-6/98	2	0	0
	Candida parapsilosis	ATCC 22019, ATCC 90018, HD2157/00, Ro3287/01a, RV-B/96	5	0	0
	Candida pelliculosa	RV-9/98	1	0	0
	Candida valida	HD1493/96, RV-4/96	2	0	0
	Saccharomyces cerevisiae	ATCC 9763, HD554/99, HD670/99, RV-A1/96	4	0	0
	Cryptococcus adeliensis	Ro3287/01b	1	0	0
	Cryptococcus albidus	HD1459/96, TIMM0351	2	0	0
	Cryptococous neoformans	ATCC 90112, ATCC 62066, ATCC 34544, EF-H23	4	0	0
	Cryptococcus uniguttulatus	HD1833/95	1	0	0
	Trichosporon asahii	HD462/97, HD2461/98, HD2626/98, HD3379/00	4	0	0
	Trichosporon cutaneum	RV-9/97, RV-3/99	2	0	0
	Trichosporon ovoides	EF-H607	1	0	0
	Rhodotorula rubra	EF-H1008, RV-III/00	2	0	0
	Exophiala dermatitidis	CBSc211, HD3681/00	2	0	0
	Sporobolomyces roseus	CBS-V1016/95	1	0	0
	Stephanoascus ciferrii	CBS-V5295/95	1	0	0
	Hansenula saturnus	HD1152/95	1	0	0
	Kloeckera apiculata	RV-8/98	1	0	0
	Aureobasidium pullulans	HD2670/99	1	0	0
	Kluyveromyces lactis	HD606/97	1	0	0
	Malassezia furfur	CBS-V7854/95	1	0	0
Molds	*Aspergillus flavus*	HD2026/98	1	0	0
	Aspergillus fumigatus	HD3482/00, HD4697/00	2	0	0
	Aspergillus nidulans	HD702/00	1	0	0
	Aspergillus niger	HD4235/00	1	0	0
	Aspergillus tamarii	ATCC 10836	1	0	0
	Aspergillus terreus	HD1884/01	1	0	0
	Fusarium oxysporum	HD3490/94	1	0	0
	Fusarium solani	CBS181.29	1	0	0
	Fusarium verticillioides	HD4274/00	0	1	0
	Penicillium chrysogenum	EF-S102	1	0	0
	Penicillium marneffei	HD2420/98	1	0	0
	Paecilomyces variotii	DSM1961	1	0	0
	Paecilomyces lilacinus	CBS430.87	1	0	0
	Scopulariopsis brevicaulis	CBSc400	1	0	0
	Geotrichum candidum	HD3227/96, RV-J9/96	0	2	0
	Sporothrix schenckii	TIMM0982	1	0	0
	Cladosporium cladosporioides	CBSc141	1	0	0
	Phialophora verrucosa	CBSc231	1	0	0
	Rhizopus oryzae	ATCC 56659	1	0	0
	Rhizomucor pusillus	HD2806/00	1	0	0
	Absidia corymbifera	ATCC 14058	1	0	0
	Mucor circinelloides	CBS192.68	1	0	0
	Cunninghamella bertholletiae	ATCC 42115	1	0	0
	Syncephalastrum racemosum	HD4509/00	1	0	0
	Neotestudina rosatii	CBSc212	1	0	0
Dermatophytes	*Microsporum canis*	RV-B/99	1	0	0
	Microsporum cookei	CBSc190	1	0	0
	Microsporum gypseum	CBSc192	1	0	0
	Trichophyton mentagrophytes	RV-B/00	1	0	0
	Trichophyton rubrum	Ro433/01	1	0	0
Dimorphic fungi	*Blastomyces dermatitidis*	TIMM 0126	1	0	0
	Histoplasma capsulatum	BA144603/00	1	0	0

[a] Strain collections: ATCC, American Type Culture Collection; B, Robert Koch Institut, Berlin, Germany; BA, Buenos Aires, R. Negroni; CBS, Centraalbureau voor Schimmelcultures, The Netherlands; DSM, Deutsche Stammsammlung für Mikroorganismen, Braunschweig, Germany; EF, Institute of Medical Microbiology and Hygiene, Erfurt, Germany; Gö, Hygiene Institute Georg-August-University, Göttingen, Germany; HD, Hygiene Institute University of Heidelberg, Germany; Ro, Department of Medical Microbiology and Hospital Hygiene, University Hospital, Rostock, Germany; RV, INSTAND: Institut für Standardisierung und Dokumentation im medizinischen Laboratorium, Düsseldorf, Germany (external quality control); TIMM, Teikyo University Institute of Medical Mycology, Japan.

[b] Measurements are given in nanograms per milliliter.

was added to 100 μl of EDTA treatment solution in 1.5-ml safe-lock Eppendorf tubes. The tubes were kept in boiling water for 3 min and centrifuged at 10,000 × *g* for 10 min. Fifty microliters of the supernatants was transferred into EB-CA1-coated wells, which contained 50 μl of horseradish peroxidase-conjugated EB-CA1. The plates were covered with adhesive film and incubated at 37°C for 90 min. After incubation, the adhesive film was removed, and the plates were washed five times each with 370 μl of washing solution. Two hundred microliters of tetramethylbenzidine chromogen solution was added to each well, and the plates were incubated in darkness at room temperature for 30 min. The enzymatic reaction was stopped by adding 100 μl of stopping solution (1.5 N sulfuric acid) in each well, and the absorbance was read at 450 and 620 nm on a microplate reader (LP400; Bio-Rad). Reactions were performed in duplicate. The mannan concentrations were calculated from the calibration curve of standard controls.

Test medium for the fungal antigen extracts. As PBS was found to lead to nonspecific false-positive results in the assay, several media, including 0.85% sodium chloride, sterile distilled water, human serum, and a series of BSA solutions ranging in concentration from 0.1 to 6%, were examined. All the protein-free solutions and the low BSA concentrations up to 2.0% yielded results that varied between 0.1 and 0.8 ng/ml and thus could not safely be considered negative. The BSA solutions with concentrations of 4.0 and 6.0% and human serum were consistently negative (<0.25 ng/ml). The 6% BSA solution was used as the diluent for the fungal antigen extracts because it resembles the protein concentration in human serum.

Assay controls. The results of the standard controls consistently fell into the range of the values given by the manufacturer. The results from the additional control of plain 6% BSA solution without fungal antigen remained below the detection level in each experiment (<0.25 ng/ml).

Reproducibility. When aliquots from one antigen extract of *C. albicans* ATCC 24433 were tested in four separate experiments, the antigen concentrations were 0.7, 0.6, 1.2, and 0.9 ng/ml (mean, 0.85 ng/ml; standard deviation, 0.23 ng/ml). Four separate antigen extracts of *C. albicans* ATCC 76615 and of *C. glabrata* RV6/97, respectively, were prepared and tested in separate experiments. The antigen concentrations of the four extracts of *C. albicans* ATCC 76615 were 0.8, 0.6, 0.9, and 0.6 ng/ml (mean, 0.73 ng/ml; standard deviation, 0.13 ng/ml). The antigen concentrations of the four extracts of *C. glabrata* RV6/97 were 1.9, 1.5, 2.1, and 2.0 ng/ml (mean, 1.88 ng/ml; standard deviation, 0.23 ng/ml).

Cross-reactivity of the PLATELIA *CANDIDA* Ag EIA. Among 63 clinically relevant fungal species, isolates of the seven species *C. albicans*, *C. dubliniensis*, *C. famata*, *C. glabrata*, *C. guilliermondii*, *C. lusitaniae*, and *C. tropicalis* led to positive results. *G. candidum* and *F. verticillioides* yielded borderline-positive to weakly positive reactions. This finding was confirmed by repeated testing of the strain HD4274/00 of *F. verticillioides* on five independent occasions. The identity of this strain was confirmed by the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. Antigen preparations from 54 other fungal species did not lead to detectable amounts of mannan (Table 1).

The PLATELIA *CANDIDA* Ag EIA was developed for the detection of circulating *Candida* mannan antigen in the bloodstream of patients suffering from invasive candidiasis. The test is based on the use of a monoclonal antibody, EB-CA1, which recognizes a mannopentose epitope of *C. albicans*. This epitope is present in large amounts on numerous mannoproteins in the cell wall of *C. albicans*. It has also been found in large amounts on mannoproteins of *C. glabrata*, *C. tropicalis*, and *C. guilliermondii* but has only been found to a lesser extent on *C. krusei*, *C. kefyr*, and *C. parapsilosis* (2, 12). As invasive candidiasis is caused more and more by nonalbicans species (7, 8), an ideal antigen test would cross-react with a wide range of different *Candida* species but would remain negative with the antigens from other clinically relevant fungi. Therefore, we checked the cross-reactivity of the PLATELIA *CANDIDA* Ag EIA by subjecting BSA solutions spiked with antigen extracts from a wide variety of fungi to this assay. The test proved to be acceptably reproducible in our hands—one individual antigen extract as well as separately prepared antigen extracts from one fungal strain showed very similar results upon separate, independent testing. We recorded positive results with preparations from seven different *Candida* species, including the most prevalent pathogenic species—*C. albicans*, *C. glabrata*, and *C. tropicalis*.

In contrast to Jacquinot et al., who reported weak cross-reactivities of EB-CA1 with *C. parapsilosis* and *C. krusei* when Western blot analysis was used (2), we were unable to measure antigens from the five strains of *C. parapsilosis* and the four strains of *C. krusei* that we tested. Two clinical studies (10, 13) support our finding of weak cross-reactions of these *Candida* species. They reported a sensitivity for the detection of mannanemia of 63 to 78% for infections caused by *C. albicans*, *C. glabrata*, and *C. tropicalis*, while that for *C. parapsilosis* and *C. krusei* was only 30 to 44%.

One strain of *F. verticillioides* and two strains of *G. candidum* repeatedly led to borderline-positive results. Antigen preparations from *Fusarium* species other than *F. verticillioides* did not cross-react. The reactivity of the PLATELIA *CANDIDA* Ag EIA with serum samples from patients with invasive fusariosis is unknown.

In conclusion, the PLATELIA *CANDIDA* Ag EIA may be used for the detection of circulating antigens of *C. albicans*, *C. dubliniensis*, *C. famata*, *C. glabrata*, *C. guilliermondii*, *C. lusitaniae*, and *C. tropicalis*. We were unable to record a reactivity of the assay with antigens from *C. parapsilosis* and *C. krusei*. Weak cross-reactions appear to be possible with *Geotrichum* and *Fusarium*, which rarely cause invasive infections. No false-positive results were caused by *Cryptococcus* or *Aspergillus*, which represent other common invasive fungal pathogens.

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