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Evaluation of Latex Reagents for Rapid Identification of *Candida albicans* and *C. krusei* Colonies

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A total of 322 yeast strains and yeastlike organisms belonging to the genera *Candida*, *Cryptococcus*, *Geotrichum*, *Saccharomyces*, and *Trichosporon* were tested with the new monoclonal antibody-based Bichro-latex albicans and Krusei color latex tests. Comparison of results with those obtained by conventional identification methods showed 100% sensitivity for both latex tests and 100% and 95% specificity for the Bichro-latex albicans and Krusei color tests, respectively. Because the test is easy to read and quick to perform, the Bichro-latex albicans test may be useful for rapid identification of *Candida albicans* colonies in the clinical laboratory.

The widespread use of broad-spectrum antibiotics and the rising number of immunocompromised patients are the major reasons leading saprophytic yeasts to become pathogenic and contributing to the emergence of new opportunistic yeasts (1, 20, 44). Moreover, opportunistic yeasts demonstrate various degrees of in vivo and in vitro resistance to common antifungal agents (21, 29), and it is now well known that strains of *Candida lusitaniae* (19) and *Candida krusei* and *Candida glabrata* (17, 20, 44) are relatively resistant to amphotericin B and fluconazole, respectively. However, *Candida albicans* remains the most common species isolated, representing between 60 and 80% of all clinical isolates of yeasts and exhibiting a high sensitivity to antifungal agents (11, 17, 30). Consequently, mycoses are a growing medical problem requiring prompt diagnosis and early antifungal therapy.

Various techniques are available for the prompt identification of *Candida albicans* (17). The germ tube (GT) test is a simple, rapid, efficient, and economical test that is available to laboratory practitioners for screening and identification of *C. albicans* (26, 41). The ability of *C. albicans* to produce GT is affected by different laboratory conditions (11, 25, 34), and an inexperienced laboratory technician may either obtain up to 5% GT-negative *C. albicans* strains or confuse germination of arthroconidia and attachment of blastospores with GT production of *C. albicans* (11, 17). Recently the detection of enzymatic activities (9, 31) has become a valuable tool either for the rapid identification of *C. albicans* by tests such as Albistrip (Lab M. Ltd., Bury, United Kingdom) (9), Murex C. albicans CA50 (Murex Diagnostics, Norcross, Ga.) (11), Rapidec albicans (bioMérieux, Marcy l'Etoile, France) (15), and Fongiscreen 4H (Sanofi Diagnostics Pasteur, Marnes la Coquette, France) (14) or for detection with direct identification with fluorogenic or chromogenic media such as Fluoroplate Candida agar (Merck Clévenot, Nogent sur Marne, France) (13, 27, 35, 43), MUAG (Biolife, Italy) (43), Albicans ID (bioMérieux) (10, 25, 35), Candichrom albicans (International Microbio, Signes, France) (42), Candiselect (Sanofi Diagnostics Pasteur) (17), and CHROMagar Candida (CHROMagar, Paris, France) (2, 3, 12, 30, 32, 36).

For the rapid identification of yeast colonies, immunological tests with species-specific antisera have been described, which use either polyclonal (38, 40) or monoclonal antibodies (4, 8, 18, 23, 28, 33, 39), but these methods either were limited by the lack of specificity of some sera, especially for the species *C. albicans* and *Candida tropicalis* (5), or were not commercially available. Recently, new monoclonal antibody-based latex tests have been proposed (Fumouze Diagnostics, Asnières, France) for the rapid identification of *C. albicans* (34) and *C. krusei* colonies. Thus, the aim of this study was to evaluate in comparison with our standard identification method the performance of these new tests with recent clinical isolates, reference strains, and *C. albicans* strains selected for their inability or poor ability to utilize specific enzyme substrates on different chromogenic media. The results showed the good ability of the Bichro-latex albicans test for rapid and specific identification of *C. albicans* colonies and, in some instances, the relative lack of specificity of the Krusei color test.

MATERIALS AND METHODS

Strains. A total of 322 yeast strains and yeastlike organisms belonging to the genera *Candida*, *Cryptococcus*, *Geotrichum*, *Saccharomyces*, and *Trichosporon* were examined, including 124 stored clinical isolates, 4 reference strains, and 194 recent clinical isolates (Table 1).

The 124 stored clinical isolates were identified by conventional methods as previously described (37) in the reference mycology laboratory of the Institut Pasteur de Lyon, Lyon, France, and stored for 2 years on Sabouraud agar at laboratory temperature. The reference identification methods included tests for carbohydrate fermentation and assimilation, cycloheximide resistance, nitrate assimilation, growth at 37°C, urease production on a Christensen urea agar slant, and tetrazolium reduction. When necessary, organisms were checked for formation of ascospores and morphology on Dalmau plates.

Four reference strains were used, namely *C. albicans* ATCC 10231 and 60193 and *C. tropicalis* ATCC 66029 from the American Type Culture Collection, Rockville, Md., and *C. albicans* IP 1274 from the Institut Pasteur collection, Paris, France.

The 194 clinical isolates were recently obtained from various clinical specimens received at our laboratory. They were isolated on Albicans ID (bioMérieux), incubated at 30°C for 24 to 48 h, and stored on Sabouraud agar. Among them eight strains of *C. albicans* were included in this study for their inability or poor ability to utilize specific enzyme substrates on Albicans ID, CHROMagar, and/or Candiselect (Table 2).

Before being tested, all strains were subcultured on Sabouraud glucose agar to check their purity, and then they were tested according to the manufacturer's instructions. If a latex test result did not agree with the result of the conventional identification method, the latex test was repeated. If the discrepancy persisted, the isolate was tested again with the API32C yeast identification system.

Latex tests. The Bichro-latex albicans (Fumouze Diagnostics) test is performed with two different reagents: a dissociating agent containing enzymes and a brown latex reagent. This latex reagent is made of red latex particles in suspension in a green dye; the particles are coated with a monoclonal antibody that specifically reacts with a *C. albicans* antigen mainly located in the cell wall. A positive reaction is traduced by red agglutinates on a green background. The presence of no agglutinates or the presence of white or red aggregates without green background is considered to be negative.

The Krusei color test (Fumouze) is performed with red latex particles coated

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TABLE 1. Origin of the strains and results of agglutination with Bichro-latex and Krusei color tests

Species	No. of:				Result by test[a]:			
	Isolates	Stored isolates	Reference strains	Total isolates	Bichro-latex albicans		Krusei color	
					+	−	+	−
Candida albicans	68	16	3	87	87	0	0	87
Candida catenulata	1			1	0	1	0	1
Candida famata	4	1		5	0	3 + (2)	2	3
Candida glabrata	28	19		47	0	47	1	46
Candida guilliermondii		4		4	0	3 + (1)	1	3
Candida inconspicua	3	3		6	0	6	1	5
Candida kefyr	19	4		23	0	23	6	17
Candida krusei	23	10		33	0	32 + (1)	33	0
Candida lipolytica	3	6		9	0	9	0	9
Candida lusitaniae	5	5		10	0	10	0	10
Candida norvegensis		2		2	0	2	0	2
Candida parapsilosis	4	11		15	0	14 + (1)	2	13
Candida pelliculosa		1		1	0	1	0	1
Candida rugosa		2		2	0	2	0	2
Candida sphaerica	1	1		2	0	(2)	2	0
Candida tropicalis	18	21	1	40	0	39 + (1)	1	39
Candida utilis		2		2	0	2	0	2
Candida zeylanoides	1			1	0	1	0	1
Cryptococcus neoformans		1		1	0	1	0	1
Geotrichum candidum	1	4		5	0	5	0	5
Saccharomyces cerevisiae	15	8		23	0	23	0	23
Trichosporon beigelii		3		3	0	3	0	3
Total	194	124	4	322	87	235	49	273

[a] Values in parentheses represent numbers of strains with agglutinates but on an unchanged brown background.

with a monoclonal antibody that specifically reacts with a *C. krusei* antigen located on the cell surface. The appearance of large red agglutinates is considered to be a positive reaction, and the appearance of no agglutinates or white agglutinates is considered to be a negative one.

RESULTS

Comparison of results from the Bichro-latex albicans and Krusei color tests with those obtained by conventional identification methods showed that 0 and 16 (5%) strains, respectively, had discrepant false-positive results. When the latex tests and the identification were repeated, these discrepant results were confirmed. Overall, the two latex tests did not show any false-negative results (Table 1).

With the Bichro-latex albicans test, eight strains showed nonspecific agglutinations, since agglutinates appeared but the background kept its original brown color, in contrast with red-specific agglutinates on a green background. These nonspecific agglutinations were obtained with 2 of 5 *Candida famata* isolates, 1 of 33 *C. krusei* isolates, 2 of 2 *Candida sphaerica* isolates, 1 of 4 *Candida guilliermondii* isolates, 1 of 15 *Candida parapsilosis* isolates, and 1 of 40 *C. tropicalis* isolates. The seven non-*C. krusei* strains showing nonspecific agglutinations with the Bichro-latex albicans test also agglutinated with the Krusei color test. Finally, the eight *C. albicans* strains selected for negative or weakly positive specific enzyme results were all unambiguously positive according to the Bichro-latex albicans test (Table 2).

The 16 non-species-specific Krusei color agglutinations were obtained with 6 of 23 *Candida kefyr* isolates, 1 of 6 *Candida inconspicua* isolates, 2 of 15 *C. parapsilosis* isolates, 2 of 2 *C. sphaerica* isolates, 2 of 5 *C. famata* isolates, 1 of 4 *C. guilliermondii* isolates, 1 of 47 *C. glabrata* isolates, and 1 of 40 *C. tropicalis* isolates.

TABLE 2. Comparison of enzymatic identification on chromogenic media with that by latex agglutination for eight *C. albicans* strains

Strain	Result by test[a]:						
	Albicans ID1		Candiselect		CHROMagar		Bichro latex albicans
	24 h	48 h	24 h	48 h	24 h	48 h	
G6837	−	−	−	−	(+)	+	+
G6848	−	−	−	−	(+)	+	+
D1356	−	−	−	−	−	−	+
G6841	−	−	−	−	(+)	+	+
8807094	−	(+)	−	(+)	(+)	+	+
8510011	(+)	+	(+)	+	(+)	+	+
361121	+	+	+	+	(+)	+	+
313117	+	+	−	(+)	+	+	(+)

[a] (+), weak.

DISCUSSION

The GT test is a very reliable, rapid, and popular procedure for the identification of *C. albicans*. Moreover, the GT test is very cheap, since the cost of a single test is no more than $0.40, including costs for supplies but not including labor costs (11, 17), and thus the small numbers of false-negative or false-positive results can be advantageously reduced by appropriate training of personnel. However, considering the increasing interest in medical mycology by nonspecialized medical laboratories, the Bichro-latex albicans test showed 100% sensitivity and specificity rates. Indeed the eight agglutinations obtained with non-*C. albicans* strains could be easily differentiated from a positive result, since the background remained brown instead of turning green as for a positive result.

Previous studies mentioned species-specific *C. albicans* components (6, 7, 16, 22), but they were not present on the blastospore surface and required a more or less complex extraction

technique. Bruneau et al. (7) previously indicated an easy enzymatic extraction procedure of components with a preparation from the intestinal juice of *Helix pomatia* and allowing the differentiation of antigenically closed yeasts such as *C. albicans*, *C. tropicalis*, and *C. parapsilosis*. However, the identification step was performed with labor-intensive and time-consuming quantitative immunoelectrophoreses. The agglutination test is a simple procedure which has been applied for many years to the study of yeast surface antigens (39). Several monoclonal antibodies have been proposed for the identification of *C. albicans* (4, 18, 23, 28, 33, 39); however, the expression of their antigenic epitopes at the surface of the yeast cell was shown to be extremely variable, and they were unable to agglutinate *C. albicans* cells with sufficient sensitivity and specificity. Thus, the Bichro-latex albicans test is the first immunological reagent useful for the rapid, easy to perform, and reliable identification of *C. albicans* colonies. Moreover, this latex test could be performed directly on blood culture bottles without the need for subculture on agar media (24), allowing specific and rapid identification of *C. albicans* fungemia.

Nonspecific agglutinations of the Bichro-latex albicans test were due to yeast agglutinates without the full cooperation of the sensitized latex particles, since the agglutinates were observed on a background remaining brown. Unfortunately, this differentiation between nonspecific agglutination of yeast cells with the specific agglutination including sensitized latex particles could not be made for the Krusei color test, since the background was not colored, in contrast with the Bichro-latex albicans test. Thus, it is possible that the false-positive results obtained with the Krusei color test were also, at least in some instances, due to nonspecific yeast agglutinations, since all seven nonspecific Bichro-latex albicans strains did show a Krusei color agglutination. These strains also showed autoagglutinations when the colonies were suspended in a drop of distilled water (data not shown). Thus, to prevent some difficulties in differentiation between autoagglutinations and specific agglutinations with the Krusei color test, control of the correct dissociation of the yeasts before testing or introduction of a green background should be recommended.

The Bichro-latex albicans test could be considered superior to the chromogenic identification media for the identification of *C. albicans*, since no false-negative results were observed with those strains selected for their inability or poor ability to utilize specific enzyme substrates on different chromogenic media (Table 2). Moreover, at least 60% of *C. albicans* strains required 48 or 72 h of incubation on chromogenic media in order to be correctly identified (2, 25, 30). However, the chromogenic media are able to facilitate the differentiation of species in mixed cultures and, at least for CHROMagar, allow direct and rapid identification of other opportunistic strains of *Candida* such as *C. tropicalis* and *C. krusei*, which are obvious advantages over the latex test (2, 25, 30, 36).

Thus, in order to instigate the right treatment without delay, we concluded that (i) the Bichro-latex albicans test may be useful in the clinical laboratory for rapid identification of *C. albicans*, especially from nonselective media such as blood agar or from blood culture bottles, and (ii) the Krusei color test, even with a perfect specificity, could not be considered very useful, since for any non-*C. albicans* yeast strain isolated from deep samples or from immunocompromised patients, the sensitivity to antifungal agents must be determined to avoid resistance, and in the meantime, a broad-spectrum agent must be prescribed (19–21, 29).

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