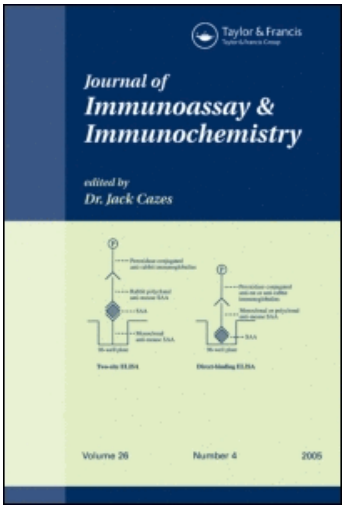


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# Rapid Identification of *Candida Albicans* by Dot-Enzyme Immunoassay

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# RAPID IDENTIFICATION OF *CANDIDA ALBICANS* BY DOT-ENZYME IMMUNOASSAY

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## ABSTRACT

A highly sensitive and specific dot-enzyme immunoassay for the rapid identification of *Candida albicans* was developed using a murine monoclonal antibody (Mab), which adsorbed to cell surface-exposed determinants. This Mab reacted with 28 of the 28 *C. albicans* strains tested including the serotypes A and B and 2 *C. stellatoidea*. It did not react with 32 other isolates representing eight other *Candida* species commonly encountered in human materials. All the test could be performed in four steps in less than an hour. The yeasts were directly spotted on a strip of Immunodyne membrane. Then the strip was incubated for 5 min. with the Mab, for 15 min. with a peroxydase-conjugate and for 30 min. with the enzyme substrate and 4-chloro 1 naphtol. This test proved useful for rapid and easy identification of *C. albicans*. (KEY WORDS : *Candida albicans*, Dot-enzyme immunoassay)

## INTRODUCTION

Infection due to fungi are of increasing importance in patients with compromised host defenses and the mortality rate associated with these infections is very high. *C. albicans* and *C. tropicalis* are the species most commonly isolated (1). The microbiological diagnosis, based upon biochemical tests, is still time consuming (2) in spite of the development of miniaturized kits (3, 4) and of automated system (5, 6). Slide agglutination tests with species-specific antisera were limited by the

lack of specificity of sera since *C. albicans* serotype A and *C. tropicalis* could not be distinguished (7, 8). A commonly used test for *C. albicans*, the "germ tube" test, required three hours and occasional isolates may give a false negative test.

Thus the laboratory needs a fast and sensitive technique to rapidly identify *C. albicans*. We report here the use of a monoclonal antibody in a dot-enzyme immunoassay for the rapid detection of this microorganism.

## MATERIALS AND METHODS

### Yeasts strains

A total of 60 yeasts or yeast-like fungi belonging to the genera *Candida*, *Saccharomyces* and *Geotrichum* were examined (Table 1). Most strains have been isolated from pathological products on Sabouraud Chloramphenicol Agar (Diagnostics Pasteur, Marne La Coquette, France). They were identified by classical criteria according to van der Walt and Yarrow (2). The other strains were stock cultures obtained from Centraalbureau voor Schimmelcultures (CBS, Delft, The Netherlands), from National Institute of Health (NIH, Bethesda, USA) or from Institut Pasteur (IP, Paris, France). For all studies yeasts were cultured on Sabouraud Chloramphenicol Agar for 24 H at 30°.

### Dot enzyme immunoassay

All the steps were performed at room temperature.

Step 1 : The blastospores or the arthrospores were removed from the surface of the agar plate and directly spotted on Immunodyne membrane (Pall Industries, St-Germain-en-Laye, France). Ten strains

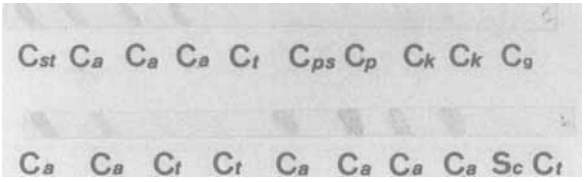


**Figure 1**

24 H old cultures from : *Candida albicans* (Ca), *C. guilliermondii* (Cg), *C. krusei* (Ck), *C. parapsilosis* (Cp), *C. pseudotropicalis* (Cps), *C. stellatoidea* (Cst), *C. tropicalis* (Ct) and *Saccharomyces cerevisiae* (Sc) were directly spotted on Immunodyne membrane. After incubation with the Mab, the peroxydase conjugate and substrate, spots from *C. albicans* were strongly blue colored. All other spots were not detected.



were spotted on a strip of 5 x 100 mm. Then the strips were soaked in Tris buffered saline pH 7.5 (TBS : Tris 20 mM, NaCl 500 mM) containing 3 % (V/V) gelatin (Biorad Laboratories, Richmond, USA) for 1 min, to block the remaining sites.

Step 2 : Incubation for 5 min with the Mab (MAB 806, Chemicon, El Segundo, California, USA) diluted 1 : 200 in TBS containing 0.1 % Tween 20 (TBST, Merck, Darmstadt, FRG) followed by two washing in TBST for 1 min. each.

Step 3 : Incubation for 15 min with peroxydase conjugated rabbit anti-mouse Ig (Ref. P 161, Dakopatts, Copenhagen, Denmark) diluted 1 : 200 in TBST.

Step 4 : After three washing in TBST for 1 min. and one in TBS for 1 min. the blots were revealed for 30 min in solution of TBS containing 0.03 % $H_2O_2$ (Merck, Darmstadt, FRG) and 0.05 % 4-chloro-1 naphtol (Sigma Chimie SA, La Verpillière, France).

**Table 1**
**Results of yeasts identification in Dot-enzyme immunoassay**

| Tested strains | Number | Positive results | Negative results |
|---|---|---|---|
| *C. albicans* 792 NIH, 311 NIH, 1431, 1422, 1543, 1189, 1513, 184, 34, HM, 1565, 1434, 1583, 5346, 1285, DUR, 1468, 1485, 214, 243, 740, GIN, BAY, 1309, MOR, BER | 26 | **26** | 0 |
| *C. albicans* received as *C. stellatoidea* CBS 1905, CBS 2990 | 2 | **2** | |
| *C. glabrata* IP 810, IP 811, 5090, 2036 | 4 | 0 | 4 |
| *C. guilliermondii* IP 624, 206 | 2 | 0 | 2 |
| *C. krusei* 573 CBS, 208 IP | 2 | 0 | 2 |
| *C. macedoniensis* CBS 600 | 1 | 0 | 1 |
| *C. parapsilosis* IP 45, IP 83, 2147 | 3 | 0 | 3 |
| *C. pseudotropicalis* CBS 607, IP 42, 1020 | 3 | 0 | 3 |
| *C. tropicalis* CBS 94, IP 43, 104, 5025, 9763, 4620, 5476, 1079, 1559, 4881, 4439, 1325, 1831 | 13 | 0 | 13 |
| *S. cerevisiae* IP 605, IP 635, 1248 | 3 | 0 | 3 |
| *G. candidum* 1065 | 1 | 0 | 1 |

## RESULTS

To evaluate the reactivity of the Mab with *C. albicans* we tested a panel of *fungi* frequently encountered in human materials. A strong blue colour corresponded to a positive reaction easy to read (Figure 1). All the 28 *C. albicans* strains were detected including the two serotypes A and B and the sucrose negative variant *C. stellatoidea*. None of the other 32 yeast strains reacted with the Mab even *C. tropicalis* which shared many properties with *C. albicans* (Table 1). All the test could be performed in less than an hour.

## DISCUSSION

A total of 60 yeasts or yeast-like strains belonging to the genera *Candida*, *Saccharomyces* and *Geotrichum* were examined. They represented the 8 *Candida* species the most often isolated from human clinical materials and known to be virulent species. *G. candidum* and *S. cerevisiae* were frequently encountered in clinical material as commensal.

Using the dot-enzyme immunoassay we obtained 100 % specificity and 100 % sensitivity for the identification of *C. albicans*. None of the other *C. albicans* identification method actually used (biochemical, serological or morphological) showed such a specificity and sensitivity. Particularly, no false positive results were observed with *C. tropicalis*. *C. albicans* and *C. tropicalis* are 2 related species showing identical DNA G + C % contents (9), and more than 80 % of cross-reacting antigens in quantitative immunoelectrophoresis (10).

This test is rapid to perform : 60 min., versus 3 h for the germ-tube test, the fastest identification method currently available. An

important point is the yeast fixation on the Immunodyne strip which is quick and strong for all strains studied. The protocol could be shortened by using an enzyme labeled Mab.

This test is easy to perform and results are read by visual observation, which eliminates the need for an ELISA plate reader or a fluorescent microscope. The development of rapid, sensitive techniques such as this for the detection of C. *albicans* infections will result in earlier treatment. In addition this Dot-ELISA test has the potential of being used for direct C. *albicans* detection in clinical swabs.



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