

## Comparative Evaluation of Macrodilution and Chromogenic Agar Screening for Determining Fluconazole Susceptibility of *Candida albicans*

THOMAS F. PATTERSON,<sup>1\*</sup> WILLIAM R. KIRKPATRICK,<sup>1</sup> SANJAY G. REVANKAR,<sup>1</sup>  
ROBERT K. MCATEE,<sup>1</sup> ANNETTE W. FOTHERGILL,<sup>2</sup> DORA I. MCCARTHY,<sup>2</sup>  
AND MICHAEL G. RINALDI<sup>1,2</sup>

Departments of Medicine<sup>1</sup> and Pathology,<sup>2</sup> The University of Texas Health Science Center at San Antonio,  
San Antonio, Texas 78284

Received 9 July 1996/Returned for modification 5 August 1996/Accepted 16 September 1996

**A simple screening method for fluconazole susceptibility using CHROMagar Candida with fluconazole was compared with the National Committee for Clinical Laboratory Standards (NCCLS) macrobroth method. In this agar dilution method, susceptible *Candida albicans* colonies are smaller on medium with fluconazole than on fluconazole-free medium. Yeasts with decreased susceptibility have normal-sized colonies on medium containing fluconazole. On agar with 16 µg of fluconazole per ml, 32 of 34 strains with NCCLS MICs of ≥16 µg/ml were correctly predicted, as were 66 of 68 with MICs of <16, an agreement of 96%. On agar with 8 µg of fluconazole per ml, 38 of 41 isolates with MICs of ≥8 were correctly predicted, as were 59 of 61 isolates with MICs of <8, an agreement of 95%. This agar dilution method appears to highly correlate with NCCLS macrobroth methods for detection of *C. albicans* and may be an effective screen for fluconazole susceptibility.**

With the widespread use of fluconazole in treating and preventing yeast infections, detection and management of yeasts with decreased fluconazole susceptibility is becoming increasingly important (5, 9, 12, 13, 15). A rapid and reproducible method for detecting fluconazole susceptibility would be useful in determining the epidemiology of and optimal treatment for clinically resistant isolates (1, 2, 4, 11, 14).

A standardized macrobroth technique for determining yeast susceptibility to fluconazole has been proposed and tested (2, 6). However, this technique requires considerable time and expense, and even microdilution modifications are not easily applicable for screening purposes (1). A newly developed chromogenic medium, CHROMagar Candida (3, 7, 8, 10), can differentiate species of *Candida* on the basis of color. We developed a screening susceptibility method by adding fluconazole to CHROMagar, which allows detection of yeasts with decreased fluconazole susceptibility (8). In this study, chromogenic agar screening was compared with macrodilution for determining fluconazole susceptibility of *Candida albicans*.

(This study was presented in part at the 96th General Meeting of the American Society for Microbiology, New Orleans, La., 19 to 23 May 1996 [abstract F86].)

**Materials and methods.** CHROMagar Candida (Paris, France) was prepared from a powdered medium according to the manufacturer's instructions, with the addition of fluconazole to give concentrations of 8 and 16 µg/ml (8). The prepared medium, which contains chloramphenicol (0.5 g/liter) and agar (15 g/liter), was brought to a boil for 15 to 30 s to dissolve the agar and cooled to 45°C in a water bath. Fluconazole intravenous solution (2 mg/ml) (Pfizer-Roerig, New York, N.Y.) was added to the above medium at 45°C, with thorough stirring, to give a final concentration of 8 and 16 µg

of fluconazole per ml in agar. Approximately 20 ml was poured in sterile 100-mm-diameter plates and allowed to cool and harden before using. Hardened plates were stored at 4°C for up to 1 week prior to use.

Consecutive clinical samples that were submitted to the Fungus Testing Laboratory (San Antonio, Tex.) for fluconazole susceptibility determination by National Committee for Clinical Laboratory Standards (NCCLS) methodology (6) were subcultured and evaluated blindly using the agar dilution method. Purified by isolation, individual colonies from each sample were archived by placing one colony in sterile distilled water. Samples were plated on the chromogenic medium which elicits a specific color pattern for *C. albicans* (green) (7, 8). From each sample stock, a sterile 10-µl loop was used to inoculate a set of three CHROMagar plates containing 0, 8, and 16 µg of fluconazole per ml. Samples were applied to one-half of each plate. Plates were incubated at 30°C for 48 h prior to assessment of growth. Results from the fluconazole-containing media were recorded as susceptible or mycologically resistant on the basis of growth characteristics. Colonies that were visually smaller, usually pinpoint in size, on medium with fluconazole than on medium without fluconazole were recorded as susceptible (Fig. 1). Colonies that demonstrated growth that was indistinguishable on medium with or without fluconazole were recorded as mycologically resistant. Results in this study were read by one of two laboratory personnel. Agar dilution susceptibility was tested on groups of 10 to 20 isolates. Control isolates with known susceptibility were included for comparison with test samples. No variation in colony morphology was noted between experiments or between batches of agar dilution plates.

**Results and discussion.** One hundred two consecutive clinical *C. albicans* isolates were evaluated by macrobroth testing and by agar dilution. A wide range of fluconazole MICs were detected, with 41 of 102 (40%) and 34 of 102 (33%) having NCCLS MICs of ≥8 and ≥16 µg/ml, respectively (Table 1).

On CHROMagar without fluconazole (Fig. 1A), growth of the susceptible *C. albicans* (top of plate) and *C. albicans* with

\* Corresponding author. Mailing address: The University of Texas Health Science Center at San Antonio, Department of Medicine, Division of Infectious Diseases, 7703 Floyd Curl Dr., San Antonio, TX 78284-7881. Phone: (210) 567-4823. Fax: (210) 567-4670. Electronic mail address (Internet): PATTERSON@UTHSCSA.EDU.

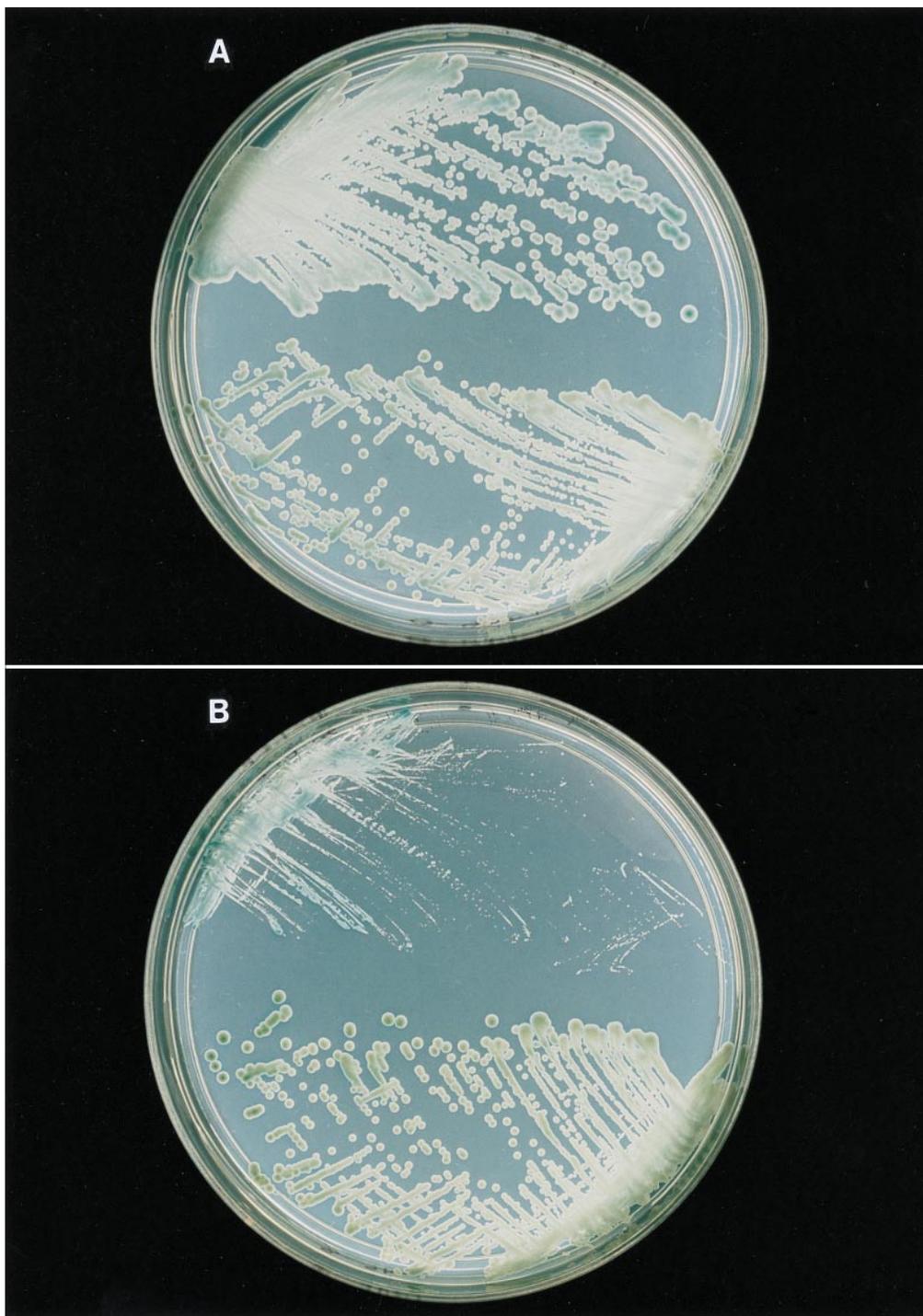


FIG. 1. (A) Susceptible *C. albicans* (top of plate) and *C. albicans* with decreased fluconazole susceptibility (bottom of plate) on CHROMagar Candida without fluconazole. (B) Susceptible *C. albicans* (top of plate) and *C. albicans* with decreased fluconazole susceptibility (bottom of plate) on CHROMagar Candida with 8  $\mu\text{g}$  of fluconazole per ml.

decreased fluconazole susceptibility (bottom of plate) could not be distinguished. Fluconazole-impregnated medium allowed distinction of *C. albicans* susceptible to fluconazole (Fig. 1, tops of plates) from *C. albicans* with decreased susceptibility (Fig. 1, bottoms of plates). The fluconazole-containing medium suppressed the susceptible strain, seen as only pinpoint

TABLE 1. Distribution of 48-h fluconazole macrobroth MICs of clinical *C. albicans* isolates tested by NCCLS macrobroth method

No. of isolates tested	No. of isolates with indicated MIC ( $\mu\text{g}/\text{ml}$ ) by NCCLS macrobroth								
	$\leq 0.25$	0.5	1	2	4	8	16	32	$\geq 64$
102	27	20	6	4	4	7	7	3	24

TABLE 2. Correlation between cultures with fluconazole susceptibility determined by NCCLS 48-h macrobroth MICs and predicted susceptibility from CHROMagar Candida with 8 µg of fluconazole per ml

Predicted CHROMagar susceptibility (µg/ml)	NCCLS macrobroth susceptibility (µg/ml)	
	MIC of <8 (n = 61)	MIC of ≥8 (n = 41)
<8	59 (97%)	3 <sup>a,b,c</sup>
≥8	2 <sup>d</sup>	38 (93%)

<sup>a</sup> One *C. albicans* isolate with a macrobroth MIC of ≥64 was predicted on CHROMagar to have a MIC of <8.

<sup>b</sup> One *C. albicans* isolate with a macrobroth MIC equal to 16 was predicted on CHROMagar to have a MIC of <8.

<sup>c</sup> One *C. albicans* isolate with a macrobroth MIC equal to 8 was predicted on CHROMagar to have a MIC of <8.

<sup>d</sup> Two *C. albicans* isolates with macrobroth MICs equal to 4 were predicted on CHROMagar to have MICs of ≥8.

colonies, whereas colonies with decreased fluconazole susceptibility were seen with normal growth characteristics.

Chromagar containing 8 µg of fluconazole per ml correctly detected 38 of 41 strains with a macrobroth MIC of ≥8 as well as 59 of 61 strains with MICs of <8 (Table 2), with agreement in 97 of 102 (95%) isolates. Agar containing 16 µg of fluconazole per ml correctly detected 32 of 34 strains with a macrobroth MIC of ≥16 and 66 of 68 strains with a MIC of <16 (Table 3), with agreement in 98 of 102 (96%). Agreement within ±1 macrobroth dilution of the screening-predicted susceptibility of ≥8 or 16 occurred in 101 of 102 isolates (99%) using CHROMagar containing 8 µg of fluconazole per ml and in 100 of 102 (98%) isolates using agar containing 16 µg of fluconazole per ml, respectively.

Normal growth on fluconazole plates containing 8 and 16 µg/ml predicted a macrobroth MIC value of at least 8 and 16 µg/ml, respectively. Sensitivity of correctly predicting decreased susceptibility by normal colony growth on medium containing 8 or 16 µg of fluconazole per ml was 93 and 94%, respectively. Specificity of predicting isolates to be fluconazole susceptible on the basis of suppressed growth on medium with fluconazole at 8 or 16 µg/ml was 97% for either dilution.

Agreement between predicted susceptibility (±1 dilution) for NCCLS MICs of less than or greater than 8 occurred in 101 of 102 (99%) isolates. One isolate using agar with fluconazole

TABLE 3. Correlation between cultures with fluconazole susceptibility determined by NCCLS 48-h macrobroth MICs and predicted susceptibility from CHROMagar Candida with 16 µg of fluconazole per ml

Predicted CHROMagar susceptibility (µg/ml)	NCCLS macrobroth susceptibility (µg/ml)	
	MIC of <16 (n = 68)	MIC of ≥16 (n = 34)
<16	66 (97%)	2 <sup>a,b</sup>
≥16	2 <sup>c,d</sup>	32 (94%)

<sup>a</sup> One *C. albicans* isolate with a macrobroth MIC of ≥64 was predicted on CHROMagar to have a MIC of <16.

<sup>b</sup> One *C. albicans* isolate with a macrobroth MIC equal to 16 was predicted on CHROMagar to have a MIC of <16.

<sup>c</sup> One *C. albicans* isolate with a macrobroth MIC equal to 8 was predicted on CHROMagar to have a MIC of ≥16.

<sup>d</sup> One *C. albicans* isolate with a macrobroth MIC equal to 4 was predicted on CHROMagar to have a MIC of ≥16.

at 8 µg/ml was predicted to be susceptible to fluconazole, but on NCCLS testing had a MIC of ≥64. All except two strains were within one dilution, using fluconazole at 16 µg/ml. One strain predicted to be susceptible on agar with fluconazole at 16 µg/ml had an NCCLS MIC of ≥64 and one strain predicted to have a MIC of ≥16 had an NCCLS MIC equal to 4.

The use of chromogenic medium with fluconazole appears to be a rapid, simple, and sensitive method for detection and identification of fluconazole-resistant *C. albicans*. Additional studies should be conducted to determine the utility of this method in screening clinical samples.

Grant support was provided by National Institutes of Health-National Institute of Dental Research 1 R01 DE11381-01, National Institutes of Health M01-RR-01346 for the Frederic C. Barter General Clinical Research Center, the Medical Research Service of the Department of Veterans Affairs, and Pfizer Inc.

Chromogenic medium was kindly provided by CHROMagar Candida (Paris, France).

#### REFERENCES

- Barchiesi, F., A. L. Colombo, D. A. McGough, and M. G. Rinaldi. 1994. Comparative study of broth macrodilution and microdilution techniques for in vitro antifungal susceptibility testing of yeasts by using the National Committee for Clinical Laboratory Standards' proposed standard. *J. Clin. Microbiol.* **32**:2494-2500.
- Fromtling, R. A., J. N. Galgiani, M. Pfaller, A. Espinel-Ingroff, K. F. Bartizal, M. S. Bartlett, B. A. Body, C. Frey, G. Hall, G. D. Roberts, F. B. Nolte, F. C. Odds, M. G. Rinaldi, A. M. Sugar, and K. Villareal. 1993. Multicenter evaluation of a broth macrodilution antifungal susceptibility test for yeasts. *Antimicrob. Agents Chemother.* **37**:39-45.
- Louwagie, B., I. Surmont, J. Verhaegen, and F. Odds. 1995. Differential and enrichment media for selective culture and recognition of yeast species from clinical material. *Eur. J. Clin. Microbiol. Infect. Dis.* **14**:406-411.
- Maenza, J. R., J. C. Keruly, R. D. Moore, R. E. Chaisson, W. G. Merz, and J. E. Gallant. 1996. Risk factors for fluconazole-resistant candidiasis in human immunodeficiency virus-infected patients. *J. Infect. Dis.* **173**:219-225.
- Millon, L., A. Manteaux, G. Reboux, C. Drobacheff, M. Monod, T. Barale, and Y. Michel-Briand. 1994. Fluconazole-resistant recurrent oral candidiasis in human immunodeficiency virus-positive patients: persistence of *Candida albicans* strains with the same genotype. *J. Clin. Microbiol.* **32**:1115-1118.
- National Committee for Clinical Laboratory Standards. 1992. Reference method for broth dilution antifungal susceptibility testing of yeasts: proposed standard. M27-P. National Committee for Clinical Laboratory Standards. Villanova, Pa.
- Odds, F. C., and R. Bernaerts. 1994. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important *Candida* species. *J. Clin. Microbiol.* **32**:1923-1929.
- Patterson, T. F., S. G. Revankar, W. R. Kirkpatrick, O. Dib, A. W. Fothergill, S. W. Redding, D. A. McGough, and M. G. Rinaldi. 1996. Simple method for detecting fluconazole-resistant yeasts with chromogenic agar. *J. Clin. Microbiol.* **34**:1794-1797.
- Pfaller, M. A., J. R. Chalberg, S. W. Redding, J. Smith, G. Farinacci, A. W. Fothergill, and M. G. Rinaldi. 1994. Variations in fluconazole susceptibility and electrophoretic karyotype among oral isolates of *Candida albicans* from patients with AIDS and oral candidiasis. *J. Clin. Microbiol.* **32**:59-64.
- Pfaller, M. A., A. Houston, and S. Coffmann. 1996. Application of CHROMagar Candida for rapid screening of clinical specimens for *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *Candida (Torulopsis) glabrata*. *J. Clin. Microbiol.* **34**:58-61.
- Redding, S., J. Smith, G. Farinacci, M. Rinaldi, A. Fothergill, J. Rhine-Chalberg, and M. Pfaller. 1994. Resistance of *Candida albicans* to fluconazole during treatment of oropharyngeal candidiasis in patients with AIDS: documentation by in vitro susceptibility testing and DNA subtype analysis. *Clin. Infect. Dis.* **18**:240-242.
- Revankar, S. G., W. R. Kirkpatrick, R. McAtee, O. P. Dib, A. W. Fothergill, S. W. Redding, D. A. McGough, M. G. Rinaldi, and T. F. Patterson. 1996. Detection and significance of fluconazole resistance in oropharyngeal candidiasis in HIV-infected patients. *J. Infect. Dis.* **174**:821-827.
- Rex, J. H., M. A. Pfaller, A. L. Barry, P. W. Nelson, and C. D. Webb. 1995. Antifungal susceptibility testing of isolates from a randomized, multicenter trial of fluconazole versus amphotericin B as treatment of non-neutropenic patients with candidemia. *Antimicrob. Agents Chemother.* **39**:40-44.
- Rex, J. H., M. A. Pfaller, M. G. Rinaldi, A. Polak, and J. N. Galgiani. 1993. Antifungal susceptibility testing. *Clin. Microbiol. Rev.* **6**:367-381.
- Rex, J. H., M. G. Rinaldi, and M. A. Pfaller. 1995. Resistance of *Candida* species to fluconazole. *Antimicrob. Agents Chemother.* **39**:1-8.