

Correlation between the procedure for antifungal susceptibility testing for *Candida* spp. of the European Committee on Antibiotic Susceptibility Testing (EUCAST) and four commercial techniques

M. Cuenca-Estrella, A. Gomez-Lopez, E. Mellado and J. L. Rodriguez-Tudela

Servicio de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain

ABSTRACT

The correlation between results obtained with the European Committee on Antibiotic Susceptibility Testing (EUCAST) antifungal susceptibility testing procedure (document 7.1) and four commercial systems was evaluated for a collection of 93 clinical isolates of *Candida* spp. Overall, agreement between the EUCAST procedure and the Sensititre YeastOne and Etest methods was 75% and 90.4%, respectively. The correlation indices ($p < 0.01$) between the EUCAST and commercial methods were 0.92 for Sensititre YeastOne, 0.89 for Etest, -0.90 for Neo-Sensitabs, and 0.95 for Fungitest. Amphotericin B MICs obtained by Sensititre YeastOne were consistently higher than with the EUCAST method and, although very major errors were not observed, 91% of MICs were misclassified. Amphotericin B- and fluconazole-resistant isolates were identified correctly with Sensititre YeastOne, Etest and Fungitest. Neo-Sensitabs identified amphotericin B-resistant isolates, but misclassified >5% of fluconazole-resistant isolates as susceptible. The commercial methods, particularly Etest and Fungitest, appeared to be suitable alternatives to the EUCAST procedure for antifungal susceptibility testing of clinical isolates of *Candida*.

Keywords Amphotericin B, antifungal testing, *Candida* spp., EUCAST, fluconazole, susceptibility testing

Original Submission: 7 September 2004; **Revised Submission:** 18 January 2005; **Accepted:** 26 January 2005

Clin Microbiol Infect 2005; 11: 486–492

INTRODUCTION

The Antifungal Susceptibility Testing Subcommittee of the European Committee on Antibiotic Susceptibility Testing (AFST-EUCAST) has developed a standard broth microdilution procedure for the determination of antifungal MICs for fermentative species of yeasts [1]. This standard is based on the NCCLS reference procedure described in document M27-A2 [2], but includes some modifications to allow for automation of the method and to permit the incubation period to be shortened from 48 to 24 h. A multicentre evaluation has demonstrated that the EUCAST procedure for antifungal

susceptibility testing is a reproducible method, with 94% agreement between laboratories [3]. In addition, a two-laboratory study evaluated the correlation between the NCCLS M27-A and EUCAST microdilution procedures with a panel of 109 bloodstream isolates of *Candida* spp., tested against amphotericin B, flucytosine, fluconazole and itraconazole, and demonstrated an overall agreement of 92% and a correlation coefficient of 0.90 ($p < 0.01$) [4]. However, standard reference procedures are generally not practical for use in routine clinical laboratories, since they involve rather complex methods for susceptibility testing. Many microbiologists prefer to use other systems with advantages such as ease of performance, economy or more rapid results. Several techniques based on agar diffusion or use of a colorimetric oxidation–reduction indicator have been developed. Some of these techniques are available commercially, and are rapid and simple alternatives to the procedures

Corresponding author and reprint requests: M. Cuenca-Estrella, Servicio de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Ctra Majadahonda-Pozuelo Km 2, 28220 Majadahonda, Madrid, Spain
E-mail: mcuenca-estrella@isciii.es

developed by either the EUCAST or NCCLS [5–8].

A significant use of reference procedures is to provide a standard from which other methods can be developed and compared. Many studies have analysed the correlation between the NCCLS procedure and various commercially available systems [5–24], including some suitable for susceptibility testing of *Candida* spp. However, only one study [25] has compared the EUCAST procedure with commercial systems. Therefore, the aim of the present study was to analyse results obtained with the EUCAST procedure and four commercially available systems for a collection of clinical isolates of *Candida* spp.

MATERIALS AND METHODS

Fungi

A collection of 93 non-duplicate clinical isolates of *Candida* spp. was tested. Most ($n = 49$) were obtained from blood cultures, while the remainder were from deep-site specimens ($n = 18$) or oropharyngeal exudates ($n = 26$). The isolates were selected to represent broad in-vitro susceptibility ranges. Each isolate was sent to the Centro Nacional de Microbiología, Madrid, Spain for identification or antifungal susceptibility testing. Isolates were identified by routine microbiological techniques (Table 1) and were maintained at -70°C . *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were used as quality control strains in each set of experiments.

Reference susceptibility testing

Standard powders of amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole and ketoconazole were supplied by Sigma Aldrich Quimica (Madrid, Spain), Pfizer (Madrid, Spain) and Janssen (Madrid, Spain). MICs were determined with the AFST-EUCAST reference procedure (document 7.1) [1]. In brief, testing was performed with RPMI-1640 medium supplemented with glucose 2% w/v, an inoculum size of 10^6 CFU/mL and flat-bottom microdilution plates [26]. MIC endpoints were determined spectrophotometrically after 24 and 48 h. For amphotericin B, the MIC endpoints were defined as the lowest drug concentration that resulted in a reduction in

growth of $\geq 90\%$ compared with that of a drug-free control well. For flucytosine and azoles, the MIC endpoint was defined as a 50% reduction in optical density.

Commercial techniques

Four commercial methods were investigated: Sensititre YeastOne panel (Trek Diagnostic Systems, East Grinstead, UK); Etest strips (AB Biodisk, Solna, Sweden) on RPMI-1640/glucose 2% w/v agar; Fungitest panel (Bio-Rad, Madrid, Spain); and the agar diffusion method with Neo-Sensitabs (A/S Rosco, Taastrup, Denmark). Susceptibility testing, reading and interpretations of the results were performed in accordance with the manufacturers' instructions. Susceptibility testing was performed in triplicate on three separate days.

Data analysis

Both on-scale and off-scale results obtained by the EUCAST reference procedure were included in the analysis. The low off-scale MICs were left unchanged, and the high off-scale MICs were converted to the next highest concentration. The reproducibility of the results obtained with the EUCAST technique and the commercial methods was evaluated by using distinct statistical tests, depending on the commercial technique investigated, as test results were expressed in different units (i.e., Sensititre YeastOne and Etest results were expressed in mg/L; Fungitest results in susceptible, intermediate and resistant categories; and Neo-Sensitabs results in inhibition (cm) diameters).

The reproducibility between the EUCAST results and MICs obtained by Sensititre YeastOne and Etest was calculated by determining the percentage of agreement between MICs. Agreement was defined as a discrepancy in MICs of no more than two doubling dilutions. Results obtained by Etest were adjusted to the nearest doubling dilution, up or down, as tested by the EUCAST method. In addition, the correlation between results was evaluated by using the intra-class correlation coefficient (ICC), which was expressed to a maximum value of 1 and with a 95% CI. In order to approximate a normal distribution, the MICs were transformed to \log_2 values. A p value of < 0.01 was considered to be statistically significant. The ICC is a reverse measurement of the variability of the counting values and was calculated using the formula $\text{ICC} = (\text{group mean square} - \text{error mean square}) / (\text{group mean square} + \text{error mean square})$; it thus has a maximum value of 1 if there is a perfect correlation and a minimum value of -1 if there is a complete absence of correlation. The ICC evaluates the correlation between values offering statistical

Table 1. Results obtained with the EUCAST procedure for *Candida* isolates included in the study

Species	MIC values (mg/L)						
	No. of isolates	Amphotericin B	Flucytosine	Fluconazole	Itraconazole	Voriconazole	Ketoconazole
<i>C. albicans</i>	21	0.03–2.0	0.12–128.0	0.12–128.0	0.01–16.0	0.01–16.0	0.01–4.0
<i>C. tropicalis</i>	21	0.03–8.0	0.06–1.0	0.12–128.0	0.01–16.0	0.01–16.0	0.01–8.0
<i>C. parapsilosis</i>	12	0.03–1.0	0.12–0.50	0.12–2.0	0.01–0.12	0.01–0.03	0.01–0.06
<i>C. glabrata</i>	10	0.06–0.25	0.12–0.25	2.0–64.0	0.25–0.50	0.03–0.50	0.06–2.0
<i>C. krusei</i>	10	0.03–0.25	2.0–16.0	32.0–128.0	0.06–0.25	0.25–1.0	0.25–1.0
<i>C. lusitanae</i>	11	0.03–1.0	0.12–0.25	0.12–64.0	0.01–0.12	0.01–0.50	0.01–1.0
<i>C. guilliermondii</i>	8	0.03–1.0	0.12–1.0	2.0–64.0	0.12–2.0	0.06–2.0	0.03–2.0
Total	93	0.03–8.0	0.12–128.0	0.12–128.0	0.01–16.0	0.01–16.0	0.01–8.0

significance, since it takes into account the number of cases and absolute value of the counting. The ICC is a scales analysis and exhibits the highest statistical power for correlation studies.

The reproducibility between the EUCAST and Neo-Sensitabs results was calculated by a simple correlation coefficient (Pearson's coefficient, r). The ICC cannot be used for correlating variables that are not expressed in the same units. A p value of <0.01 was considered to be statistically significant.

The analysis of concordance between the EUCAST and Fungitest data was performed by the *gamma* ordinal probabilistic measure. This summarises, on a -1 to $+1$ scale, the extent to which higher categories (based on the data codes used) of one variable are associated with higher categories of the second variable. Data codes used with Fungitest results were susceptible = 0, intermediate = 1, and resistant = 2.

Categorical agreement between the EUCAST data and the results obtained by the commercially available techniques was also evaluated. The EUCAST has not defined interpretative breakpoints, while the breakpoints proposed by the NCCLS for flucytosine, fluconazole and itraconazole were not used, since substantial discrepancies have been observed among fluconazole-resistant isolates as a result of the well-known tendency for MICs to rise by one or two dilutions between incubation for 24 and 48 h [27]. It should be noted that the EUCAST document recommends MIC determination at 24 h, while the NCCLS M27-A2 methodology recommends an endpoint reading after incubation for 48 h. Isolates were classified as susceptible, intermediate or resistant, based on the wild-type distribution of MICs determined by the EUCAST method, on preliminary studies of the in-vitro/in-vivo correlation for strains causing oropharyngeal candidosis in AIDS patients, and on pharmacokinetic/pharmacodynamic bibliographic data [28–34]. It should be emphasised that these breakpoints are tentative and could be changed when the AFST-EUCAST reports new susceptibility data. Tentative interpretative breakpoints were: (1) amphotericin B, susceptible ≤ 0.25 mg/L, intermediate 0.50–1.0 mg/L, and resistant ≥ 2 mg/L; (2) flucytosine, susceptible ≤ 4 mg/L, intermediate 8–16 mg/L, and resistant ≥ 32 mg/L; (3) fluconazole, susceptible ≤ 2 mg/L, intermediate 4–8 mg/L, and resistant ≥ 16 mg/L; (4) itraconazole, susceptible ≤ 0.12 mg/L, intermediate 0.25–0.50 mg/L, and resistant ≥ 1 mg/L; (5) voriconazole, susceptible ≤ 0.25 mg/L, intermediate 0.50–1.0 mg/L, and resistant ≥ 2 mg/L; and (6) ketoconazole, susceptible ≤ 0.12 mg/L, intermediate 0.25–0.50 mg/L, and resistant ≥ 1 mg/L. Similar interpretative breakpoints were used to classify Sensititre YeastOne. Interpretative criteria for Etest, Fungitest and Neo-Sensitabs were according to the manufacturers' recommendations.

Categorical agreement was defined as the percentage of isolates classified in the same category with the reference procedure and each commercial technique. Discrepancies were considered to be very major if an isolate classified as resistant by the reference method was categorised as susceptible by the commercial method. Discrepancies were considered to be major if an isolate classified as susceptible by the reference method was classified as resistant by the commercial technique. Errors were classified as minor when susceptible vs. intermediate, resistant vs. intermediate, intermediate vs. susceptible or intermediate vs. resistant discrepancies were observed [7].

All statistical analyses were performed with SPSS v. 12.0 software (SPSS, Madrid, Spain).

RESULTS

Susceptibility results obtained by the EUCAST procedure are shown in Table 1. Broad MIC ranges of each antifungal agent were observed. Four isolates (one *Candida albicans* and three *Candida tropicalis*) exhibited resistance *in vitro* to amphotericin B. Eleven isolates (four *C. albicans* and seven *C. krusei*) had flucytosine MICs that were categorised as intermediate or resistant. In total, 39 isolates (ten *C. albicans*, 11 *C. tropicalis*, two *C. parapsilosis*, two *Candida glabrata*, ten *C. krusei*, one *Candida lusitaniae* and three *Candida guilliermondii*) had fluconazole MICs ≥ 16 mg/L. In-vitro resistance to itraconazole was observed for 11 isolates (five *C. albicans*, five *C. tropicalis* and one *C. guilliermondii*). Ten isolates (three *C. albicans*, six *C. tropicalis* and one *C. guilliermondii*) had voriconazole MICs >1 mg/L. Finally, 18 isolates (four *C. albicans*, four *C. tropicalis*, two *C. glabrata*, six *C. krusei*, one *C. lusitaniae* and one *C. guilliermondii*) had resistance to ketoconazole.

Table 2 shows the MIC ranges and susceptibility data obtained for the quality control strains by each susceptibility testing method. Tables 3 and 4 show the correlation indices (ICCs, r and *gamma*) between the EUCAST results and the susceptibility results obtained with each commercial technique, grouped according to each species of *Candida* and each antifungal agent, respectively. Overall, the agreement between the EUCAST and Sensititre YeastOne data was 75%, with an ICC value of 0.92 ($p < 0.01$). The agreement between the EUCAST and Etest data was 90.4%, with an ICC value of 0.89 ($p < 0.01$). Pearson's coefficient for EUCAST and Neo-Sensitabs data was -0.90 ($p < 0.01$), indicating that the higher MIC values with the EUCAST procedure were associated with smaller inhibition diameters. The *gamma* probabilistic measure between reference method and Fungitest data was 0.95 ($p < 0.01$). The lowest percentages of agreement and correlation indices were observed with data collected by Sensititre YeastOne. Notably, agreement for amphotericin B MICs was 50.5%, with an ICC value of 0.29 (no statistical significance).

Regarding categorical agreement, Table 5 shows the very major, major and minor discrepancies

Table 2. MIC ranges and susceptibility data (30 repetitions on different days) for quality control strains grouped according to susceptibility testing method

Quality control strain	Method ^a	Amphotericin B	Flucytosine	Fluconazole	Itraconazole	Voriconazole	Ketoconazole
<i>C. krusei</i> ATCC 6258	EUCAST	0.12–0.50	1.0–4.0	8.0–32.0	0.06–0.25	0.06–0.25	0.06–0.25
	YeastOne	0.50–2.0	4.0–8.0	8.0–64.0	0.12–0.50	ND	0.12–0.50
	Etest	0.50–1.0	8.0–32.0	32.0–256.0	0.12–0.50	0.12–0.50	0.12–0.50
	Neo-Sensitabs	21.0–23.2	14.2–18.8	11.8–18.8	22.5–24.1	ND	23.1–30.4
<i>C. parapsilosis</i> ATCC 22019	Fungitest ^b	< 2.0	2.0–32.0	8.0–64.0	< 0.50	ND	< 0.50–4.0
	EUCAST	0.12–0.50	0.12–0.50	1.0–4.0	0.03–0.12	0.01–0.06	0.01–0.06
	YeastOne	0.25–1.0	0.06–0.25	0.50–4.0	0.12–0.50	ND	0.03–0.12
	Etest	0.25–1.0	0.12–0.50	2.0–8.0	0.06–0.12	0.03–0.12	0.03–0.12
	Neo-Sensitabs	23.1–25.6	40.5–47.8	34.7–38.6	20.5–24.2	ND	35.5–40.6
	Fungitest ^b	< 2.0	< 2.0	< 8.0	< 0.50	ND	< 0.50

ND, Not done.

^aEUCAST, YeastOne, Etest and Fungitest values are in mg/L, while Neo-Sensitabs data are inhibition diameters in mm.

^bFungitest susceptibility data are expressed according to the two different concentrations of antifungal agents that are included in the commercial kit.

Table 3. Agreement values and correlation coefficients between EUCAST and commercial technique data, grouped according to *Candida* spp.

Species	Sensititre YeastOne		Etest		Neo-Sensitabs Pearson's coefficient, <i>r</i> ^a	Fungitest <i>Gamma</i> measure
	Agreement (%)	ICC	Agreement (%)	ICC		
<i>C. albicans</i>	90.0	0.91 ^b	92.5	0.86 ^b	– 0.93 ^b	0.96 ^b
<i>C. tropicalis</i>	56.1	0.69 ^b	88.2	0.81 ^b	– 0.91 ^b	0.94 ^b
<i>C. parapsilosis</i>	88.2	0.88 ^b	91.2	0.80 ^b	– 0.92 ^b	0.95 ^b
<i>C. glabrata</i>	69.1	0.76 ^b	87.5	0.76 ^b	– 0.89 ^b	0.88 ^b
<i>C. krusei</i>	74.0	0.88 ^b	91.4	0.88 ^b	– 0.90 ^b	0.88 ^b
<i>C. lusitanae</i>	72.9	0.81 ^b	86.8	0.74 ^b	– 0.91 ^b	0.95 ^b
<i>C. guilliermondii</i>	75.0	0.85 ^b	89.4	0.82 ^b	– 0.92 ^b	0.96 ^b

ICC, intraclass correlation coefficient.

^aPearson's coefficient between EUCAST MIC values and inhibition diameters in cm.

^b*p* < 0.01.

Table 4. Agreement values and correlation coefficients between EUCAST and commercial technique data, grouped according to antifungal agent

Species	Sensititre YeastOne		Etest		Neo-Sensitabs Pearson's coefficient, <i>r</i> ^a	Fungitest <i>Gamma</i> measure
	Agreement (%)	ICC	Agreement (%)	ICC		
Amphotericin B	50.5	0.29	87.1	0.83 ^b	– 0.89 ^b	0.94 ^b
Flucytosine	72.0	0.81 ^b	88.9	0.84 ^b	– 0.90 ^b	0.95 ^b
Fluconazole	90.0	0.93 ^b	91.0	0.95 ^b	– 0.93 ^b	0.95 ^b
Itraconazole	81.0	0.85 ^b	90.0	0.80 ^b	– 0.88 ^b	0.93 ^b
Voriconazole	ND	ND	92.0	0.93 ^b	ND	ND
Ketoconazole	82.0	0.91 ^b	91.0	0.94 ^b	– 0.89 ^b	0.86 ^b

ICC, intraclass correlation coefficient; ND, not done.

^aPearson's coefficient between EUCAST MIC values and inhibition diameters in cm.

^b*p* < 0.01.

Table 5. Categorical agreement between EUCAST and commercial technique data, grouped according to type of discrepancy (very major, major and minor errors) and antifungal agent

Antifungal agent	Number and percentage of discrepancies ^a											
	Sensititre YeastOne			Etest			Neo-Sensitabs			Fungitest		
	Very major	Major	Minor	Very major	Major	Minor	Very major	Major	Minor	Very major	Major	Minor
Amphotericin B	0	37 (13%)	216 (78%)	0	0	9 (3.2%)	0	0	4 (1.4%)	0	0	18 (6.5%)
Flucytosine	0	0	18 (6.5%)	0	9 (3.2%)	27 (7.5%)	0	14 (5.0%)	28 (10%)	0	0	9 (3.2%)
Fluconazole	0	0	32 (11.5%)	0	0	3 (1.1%)	15 (5.4%)	0	32 (11.5%)	0	0	64 (23%)
Itraconazole	0	0	92 (33%)	0	9 (3.2%)	46 (16.5%)	3 (1.1%)	0	63 (22.5%)	0	0	19 (7%)
Voriconazole	ND	ND	ND	0	0	10 (3.6%)	ND	ND	ND	ND	ND	ND
Ketoconazole	0	0	65 (23.5%)	0	0	46 (16.5%)	3 (1.1%)	0	37 (13%)	0	0	46 (16.5%)

ND, not done. Very major discrepancies are marked in bold type.

^aCalculated following three repetitions on different days (3 × 93 isolates = 279 values for each antifungal agent).

observed between the EUCAST and commercial techniques, grouped according to antifungal agent. Very major errors were found infrequently, but

some discrepancies should be highlighted. In total, 33% of susceptibility values obtained by Sensititre YeastOne were categorised incorrectly when

compared with the EUCAST results. The MICs obtained with the commercial technique were consistently higher (two or three two-fold dilutions) than those obtained with the EUCAST method. Very major errors were not observed, but 37 (2.6%) of 1395 determinations were classified as major errors, with 423 (30.3%) minor errors. The highest discrepancy was obtained for amphotericin B, with 253 (91%) of 279 MIC values misclassified, including 37 major and 216 minor discrepancies. Eight isolates (four *C. krusei*, three *C. tropicalis* and one *C. glabrata*), categorised as amphotericin B-susceptible by the EUCAST method, were classified as resistant *in vitro* by Sensititre YeastOne. It should be noted that agreement and correlation were particularly significant for *C. albicans* (90% and 0.91; Table 3). For other species, the agreement was <89% and the ICC value was ≤ 0.88 ($p < 0.01$).

Minor discrepancies were noted for 141 (8.4%) of 1674 Etest results, compared with the EUCAST reference method. The highest rates of disagreement were observed for itraconazole and ketoconazole MICs, particularly for isolates of *C. krusei* and *C. glabrata*, with *c.* 17% of results being at variance with reference values. Major errors were found rarely (18 (1.1%) of 1674 determinations), and resulted from the classification of some itraconazole-susceptible *C. krusei* isolates as resistant. Very major errors were not observed.

Very major errors were observed between the EUCAST and Neo-Sensitabs results. Five isolates (two *C. albicans*, two *C. glabrata* and one *C. tropicalis*), classified as fluconazole-resistant with the EUCAST method, were susceptible with the Neo-Sensitabs technique, and one *C. tropicalis* isolate, resistant to itraconazole and ketoconazole, was classified as susceptible to both agents with the commercial method. Very major errors were noted consistently after three repetitions. In addition, 14 (1%) of 1395 determinations were considered to be major errors, and 168 (11.7%) were minor errors.

Finally, neither very major errors nor major discrepancies were observed for the EUCAST and Fungitest results. Minor discrepancies were noted in 156 (11.2%) of 1395 MIC determinations, particularly for *C. krusei* and *C. glabrata* isolates, where 25% of isolates classified as resistant to fluconazole and ketoconazole with the EUCAST method were misclassified as intermediate with the commercial technique.

DISCUSSION

Commercial techniques have some advantages over reference methods. Generally, they are easier to perform, are more economical and can be used readily in clinical laboratories. However, some commercial techniques are not significantly less expensive than microdilution reference procedures, and can provide susceptibility results for amphotericin B and azole agents that are in total disagreement with those obtained with reference methods.

The present study evaluated the concordance between the EUCAST reference method and four commercial techniques, as well as the ability of the four commercial techniques to detect clinical isolates resistant *in vitro* to either amphotericin B or azole agents. Overall, the agreement and correlation with the EUCAST data were high, with correlation indices of 0.92 for Sensititre YeastOne, 0.89 for Etest, -0.90 for Neo-Sensitabs, and 0.95 for Fungitest ($p < 0.01$). However, the Sensititre YeastOne and EUCAST data were not comparable for amphotericin B, with 91% of susceptibility results obtained by the commercial technique being misclassified. Very major errors were not found, but most isolates susceptible to amphotericin B *in vitro* were categorised as intermediate with Sensititre YeastOne (216 of 279 determinations), with MICs obtained by the commercial method being two or three two-fold concentrations higher than EUCAST MICs. Similar differences have been reported between Sensititre YeastOne and NCCLS reference procedures [6,10,14], indicating that the commercial method could be unreliable for amphotericin B susceptibility testing. However, as reported previously [6,7,10,14,18], Sensititre YeastOne had a high percentage agreement and correlation index for fluconazole MICs.

Etest results generally matched EUCAST MIC values. Etest detected isolates resistant to amphotericin B and fluconazole, with no very major errors. The commercial method identified amphotericin B-resistant isolates accurately, as found previously in comparisons between the NCCLS and Etest methods [21,35,36], although the limited number of amphotericin B-resistant isolates included in the present study precludes further analysis. The lowest percentages of agreement were observed for MICs of azole agents, and for some isolates of *C. tropicalis*, *C. glabrata*, *C. krusei* and

C. lusitanae. These isolates often required incubation for 48 h, and were all subject to significant trailing growth, such that the MICs at 24 h were much lower than at 48 h. It could be argued that trailing growth may be an important source of variability and inaccuracy in MIC determination with Etest [22], but, in general, Etest appeared to be a convenient alternative method for testing the susceptibility of *Candida* spp. [5,11,13,16,17].

The only commercial technique tested that exhibited very major errors was the Neo-Sensitabs agar diffusion method, with 5.4% of isolates classified as resistant by the EUCAST procedure being misclassified as susceptible. A similar observation was made in studies that compared the NCCLS and Neo-Sensitabs methods [19,20]. These data indicate that this commercial method is not a reliable procedure for the identification of fluconazole-resistant isolates.

Fungitest and EUCAST susceptibility determinations were comparable for the five antifungal agents tested. In contrast to the other commercial methods, Fungitest does not provide MIC values, but is a colorimetric breakpoint method. Several studies have reported very major discrepancies (0.6–16.6%) between the NCCLS and Fungitest methods for fluconazole susceptibility testing [8,12,20,23,24]. However, the present study did not reveal any very major errors, and only 11.2% minor errors were detected, in agreement with a previous study that recommended Fungitest as a simple screening procedure for susceptibility testing of *Candida* [12].

In conclusion, susceptibility testing results obtained by the Sensititre YeastOne, Etest, Neo-Sensitabs and Fungitest methods were generally comparable to those obtained by the EUCAST procedure, with statistically significant correlation indices. However, amphotericin B MICs obtained by Sensititre YeastOne were consistently higher and, although very major errors were not observed, 91% of MICs were misclassified. Isolates resistant to amphotericin B and fluconazole were identified correctly by Sensititre YeastOne, Etest and Fungitest. Neo-Sensitabs identified amphotericin B-resistant isolates, but misclassified >5% of fluconazole-resistant isolates as susceptible. These commercial methods, particularly Etest and Fungitest, appear to be suitable alternatives to the EUCAST procedure for antifungal susceptibility testing of clinical isolates of *Candida*.

ACKNOWLEDGEMENTS

This work was supported in part by Izasa and Bio-Rad, Madrid, Spain.

REFERENCES

- Rodriguez-Tudela JL, Barchiesi F, Bille J *et al.* Method for the determination of minimum inhibitory concentration (MIC) by broth dilution of fermentative yeasts. *Clin Microbiol Infect*, 2003; **9**: I–VIII.
- National Committee for Clinical Laboratory Standards. *Reference method for broth dilution antifungal susceptibility testing of yeasts*. Document M27-A2. Wayne, PA: NCCLS, 2002.
- Cuenca-Estrella M, Moore CB, Barchiesi F *et al.* Multicenter evaluation of the reproducibility of the proposed antifungal susceptibility testing method for fermentative yeasts of the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antimicrobial Susceptibility Testing (AFST-EUCAST). *Clin Microbiol Infect* 2003; **9**: 467–474.
- Cuenca-Estrella M, Lee-Yang W, Ciblak MA *et al.* Comparative evaluation of NCCLS M27-A and EUCAST broth microdilution procedures for antifungal susceptibility testing of *Candida* species. *Antimicrob Agents Chemother* 2002; **46**: 3644–3647.
- Arikan S, Gur D, Akova M. Comparison of Etest, microdilution and colorimetric dilution with reference broth microdilution method for antifungal susceptibility testing of clinically significant *Candida* species isolated from immunocompromised patients. *Mycoses* 1997; **40**: 291–296.
- Espinel-Ingroff A, Pfaller M, Messer SA *et al.* Multicenter comparison of the sensititre YeastOne Colorimetric Antifungal Panel with the National Committee for Clinical Laboratory standards M27-A reference method for testing clinical isolates of common and emerging *Candida* spp., *Cryptococcus* spp., and other yeasts and yeast-like organisms. *J Clin Microbiol* 1999; **37**: 591–595.
- Morace G, Amato G, Bistoni F *et al.* Multicenter comparative evaluation of six commercial systems and the National Committee for Clinical Laboratory Standards M27-A broth microdilution method for fluconazole susceptibility testing of *Candida* species. *J Clin Microbiol* 2002; **40**: 2953–2958.
- Willinger B, Engelmann E, Hofmann H *et al.* Multicenter comparison of Fungitest for susceptibility testing of *Candida* species. *Diagn Microbiol Infect Dis* 2002; **44**: 253–257.
- Barry AL, Pfaller MA, Rennie RP, Fuchs PC, Brown SD. Precision and accuracy of fluconazole susceptibility testing by broth microdilution, Etest, and disk diffusion methods. *Antimicrob Agents Chemother* 2002; **46**: 1781–1784.
- Chryssanthou E. Trends in antifungal susceptibility among Swedish *Candida* species bloodstream isolates from 1994 to 1998: comparison of the E-test and the Sensititre YeastOne Colorimetric Antifungal Panel with the NCCLS M27-A reference method. *J Clin Microbiol* 1994; **39**: 4181–4183.
- Colombo AL, Barchiesi F, McGough DA, Rinaldi MG. Comparison of Etest and National Committee for Clinical Laboratory Standards broth microdilution method for

- azole antifungal susceptibility testing. *J Clin Microbiol* 1995; **33**: 535–540.
12. Davey KG, Holmes AD, Johnson EM, Szekely A, Warnock DW. Comparative evaluation of FUNGITEST and broth microdilution methods for antifungal drug susceptibility testing of *Candida* species and *Cryptococcus neoformans*. *J Clin Microbiol* 1998; **36**: 926–930.
 13. Espinel-Ingroff A, Pfaller M, Erwin ME, Jones RN. Inter-laboratory evaluation of Etest method for testing antifungal susceptibilities of pathogenic yeasts to five antifungal agents by using Casitone agar and solidified RPMI 1640 medium with 2% glucose. *J Clin Microbiol* 1996; **34**: 848–852.
 14. Espinel-Ingroff A, Pfaller M, Messer SA, Knapp CC, Holliday N, Killian SB. Multicenter comparison of the Sensititre YeastOne colorimetric antifungal panel with the NCCLS M27-A2 reference method for testing new antifungal agents against clinical isolates of *Candida* spp. *J Clin Microbiol* 2004; **42**: 718–721.
 15. Martin-Mazuelos E, Gutierrez MJ, Aller AI *et al.* A comparative evaluation of Etest and broth microdilution methods for fluconazole and itraconazole susceptibility testing of *Candida* spp. *J Antimicrob Chemother* 1999; **43**: 477–481.
 16. Pfaller MA, Diekema DJ, Messer SA, Boyken L, Hollis RJ. Activities of fluconazole and voriconazole against 1,586 recent clinical isolates of *Candida* species determined by broth microdilution, disk diffusion, and Etest methods: report from the ARTEMIS Global Antifungal Susceptibility Program, 2001. *J Clin Microbiol* 2003; **41**: 1440–1446.
 17. Pfaller MA, Diekema DJ, Boyken L, Messer SA, Tendolkar S, Hollis RJ. Evaluation of the Etest and disk diffusion methods for determining susceptibilities of 235 blood-stream isolates of *Candida glabrata* to fluconazole and voriconazole. *J Clin Microbiol* 2003; **41**: 1875–1880.
 18. Posteraro B, Romano L, Sanguinetti M, Masucci L, Morace G, Fadda G. Commercial systems for fluconazole susceptibility testing of yeasts: comparison with the broth microdilution method. *Diagn Microbiol Infect Dis* 2000; **38**: 29–36.
 19. Soni LM, Burattini MN, Pignatari AC, Gompertz OF, Colombo AL. Comparative study of agar diffusion test and the NCCLS macrobroth method for in vitro susceptibility testing of *Candida* spp. *Mycopathologia* 1999; **145**: 131–135.
 20. Swinne D, Raes-Wuytack C, Van Looveren K, Desmet P. Comparative evaluation of Fungitest-, Neo-Sensitabs- and M27T-NCCLS broth microdilution methods for antifungal drug susceptibility testing of *Candida* species and *Cryptococcus neoformans*. *Mycoses* 1999; **42**: 231–237.
 21. Wanger A, Mills K, Nelson PW, Rex JH. Comparison of Etest and National Committee for Clinical Laboratory Standards broth microdilution method for antifungal susceptibility testing: enhanced ability to detect amphotericin B-resistant *Candida* isolates. *Antimicrob Agents Chemother* 1995; **39**: 2520–2522.
 22. Warnock DW, Johnson EM, Rogers TR. Multi-centre evaluation of the Etest method for antifungal drug susceptibility testing of *Candida* spp. and *Cryptococcus neoformans*. BSAC Working Party on Antifungal Chemotherapy. *J Antimicrob Chemother* 1998; **42**: 321–331.
 23. Willinger B, Apfalter P, Hirschl AM, Makristathis A, Rotter M, Seibold M. Susceptibility testing of *Candida* species: comparison of NCCLS microdilution method with Fungitest. *Diagn Microbiol Infect Dis* 2000; **38**: 11–15.
 24. Witthuhn F, Toubas D, Beguinot I *et al.* Evaluation of the fungitest kit by using strains from human immunodeficiency virus-infected patients: study of azole drug susceptibility. *J Clin Microbiol* 1999; **37**: 864–866.
 25. Chryssanthou E, Cuenca-Estrella M. Comparison of the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antibiotic Susceptibility Testing proposed standard and the E-test with the NCCLS broth microdilution method for voriconazole and caspofungin susceptibility testing of yeast species. *J Clin Microbiol* 2002; **40**: 3841–3844.
 26. Cuenca-Estrella M, Diaz-Guerra TM, Mellado E, Rodriguez-Tudela JL. Influence of glucose supplementation and inoculum size on growth kinetics and antifungal susceptibility testing of *Candida* spp. *J Clin Microbiol* 2001; **39**: 525–532.
 27. Espinel-Ingroff A, Rodriguez AL, Barchiesi F *et al.* International comparison of EUCAST and NCCLS M27-A2 broth microdilution methods for testing triazoles against *Candida* spp. [abstract M-1205]. In: *Abstracts of the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago*. Washington, DC: American Society for Microbiology, 2003; 464.
 28. Andes D, van Ogtrop M. In vivo characterization of the pharmacodynamics of flucytosine in a neutropenic murine disseminated candidiasis model. *Antimicrob Agents Chemother* 2000; **44**: 938–942.
 29. Andes D, Stamsted T, Conklin R. Pharmacodynamics of amphotericin B in a neutropenic-mouse disseminated-candidiasis model. *Antimicrob Agents Chemother* 2001; **45**: 922–926.
 30. Andes D, Marchillo K, Stamstad T, Conklin R. In vivo pharmacokinetics and pharmacodynamics of a new triazole, voriconazole, in a murine candidiasis model. *Antimicrob Agents Chemother* 2003; **47**: 3165–3169.
 31. Cuenca-Estrella M, Rodero L, Garcia-Effron G, Rodriguez-Tudela JL. Antifungal susceptibilities of *Candida* spp. isolated from blood in Spain and Argentina, 1996–1999. *J Antimicrob Chemother* 2002; **49**: 981–987.
 32. Cuenca-Estrella M, Gomez-Lopez A, Mellado E, Garcia-Effron G, Rodriguez-Tudela JL. In vitro activities of voriconazole and four other antifungal agents against fluconazole-resistant or -susceptible clinical yeast isolates. *Antimicrob Agents Chemother* 2004; **48**: 3107–3111.
 33. Laguna F, Rodriguez-Tudela JL, Martinez-Suarez JV *et al.* Patterns of fluconazole susceptibility in isolates from human immunodeficiency virus-infected patients with oropharyngeal candidiasis due to *Candida albicans*. *Clin Infect Dis* 1997; **24**: 124–130.
 34. Rex JH, Pfaller MA, Walsh TJ *et al.* Antifungal susceptibility testing: practical aspects and current challenges. *Clin Microbiol Rev* 2001; **14**: 643–658.
 35. Lewis RE, Diekema DJ, Messer SA, Pfaller MA, Klepser ME. Comparison of Etest, checkerboard dilution and time-kill studies for the detection of synergy or antagonism between antifungal agents tested against *Candida* species. *J Antimicrob Chemother* 2002; **49**: 345–351.
 36. Peyron F, Favel A, Michel-Nguyen A, Gilly M, Regli P, Bolmstrom A. Improved detection of amphotericin B-resistant isolates of *Candida lusitanae* by Etest. *J Clin Microbiol* 2001; **39**: 339–342.