Evaluation of Etest Method for Determining Posaconazole MICs for 314 Clinical Isolates of Candida Species

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The performance of the Etest for posaconazole (SCH 56592) susceptibility testing of 314 isolates of Candida spp. was assessed against the National Committee for Clinical Laboratory Standards (NCCLS) microdilution broth method. The NCCLS method employed RPMI 1640 broth medium, and MICs were read after incubation for 48 h at 35°C. MICs were determined by Etest for all 314 isolates with RPMI agar containing 2% glucose (RPG agar) and were read after incubation for 48 h at 35°C. The Candida isolates included C. albicans (n = 174), C. glabrata (n = 57), C. tropicalis (n = 31), C. parapsilosis (n = 39), C. krusei (n = 5), C. guilliermondii (n = 6), and C. lusitaniae (n = 2). The Etest results correlated well with reference MICs. Overall agreement was 95%, and agreements for individual species were as follows: C. krusei, 100%; C. albicans, 98%; C. tropicalis, 97%; C. glabrata, 93%; C. parapsilosis, 85%; C. guilliermondii, 83%; and C. lusitaniae, 50%. The problem of trailing end points was minimized with RPG agar, and good agreement with broth dilution MICs was obtained when discernible growth within an established ellipse was ignored. The Etest method using RPG agar appears to be a useful method for determining posaconazole susceptibilities of Candida species.

Agar-based methods for antimicrobial susceptibility testing include agar dilution, disk diffusion, and the Etest and are used widely in clinical laboratories due to flexibility and ease of performance (9). The Etest stable agar gradient method has been shown to provide reference quality MIC determinations for a variety of pathogens and antimicrobial agents, including Candida spp. and filamentous fungi (7, 9, 12–17; M. A. Pfaller, S. A. Messer, K. Mills, A. Bolmström, and R. N. Jones, submitted for publication). Studies have shown that when performed according to the manufacturer’s instructions, the Etest provides excellent performance for testing Candida spp. against a variety of antifungal agents, including polyenes, fluconazole, and azoles (4, 6, 12–16, 18). Recent studies have demonstrated that the Etest is suitable for testing the investigational antifungal agents voriconazole and caspofungin against Candida (15; Pfaller et al., submitted).

Another investigational triazole antifungal agent, posaconazole, has potent activity against pathogenic yeasts, including most species of Candida (1, 3, 5, 8, 11, 12). This agent has been widely tested in broth according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines but has not yet been evaluated using an agar-based method. Given the success in testing other triazoles using the Etest, it is reasonable to assume that posaconazole may be tested by this method as well. The availability of a variety of Etest reagents for antifungal testing will provide great flexibility for laboratories that wish to perform quantitative antifungal susceptibility testing using selected antifungal agents.

In previous evaluations of the Etest for testing amphotericin B (14), fluconazole (13), voriconazole (15), and caspofungin (Pfaller et al., submitted), we have utilized three different media, RPMI 1640 agar supplemented with 2% glucose (RPG agar), Casitone agar, and Antibiotic Medium 3 agar. In every instance we have found performance to be best with RPG agar. Thus, in the present study we evaluated the Etest for posaconazole using only RPG agar in comparison to the NCCLS reference microdilution broth method for testing 314 clinical isolates of Candida spp.

MATERIALS AND METHODS

Test organisms. Three hundred fourteen clinical isolates of Candida species were selected for testing. The collection included 174 Candida albicans, 57 Candida glabrata, 39 Candida parapsilosis, 31 Candida tropicalis, six Candida guilliermondii, five Candida krusei, and two Candida lusitaniae isolates. The members of this collection were all recent clinical isolates from geographically diverse medical centers in North and Latin America. The majority were isolated from blood or normally sterile body fluids (12). The isolates were identified by standard methods (19) and were stored as suspensions in water at ambient temperature until used in the study. Prior to testing, each isolate was subcultured at least twice onto potato dextrose agar (Remel, Lenexa, Kans.) to ensure optimal growth characteristics.

Antifungal agents. Etest strips containing posaconazole were supplied by AB BIODISK (Solna, Sweden). Posaconazole was obtained as a powder from Schering-Plough Research Institute (Kenilworth, N.J.). Stock solutions were prepared in polyethylene glycol. Serial twofold dilutions were prepared exactly as outlined in NCCLS document M27-A (10). Final dilutions were made in RPMI 1640 medium buffered to pH 7.0 with 0.16 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma). The final concentration of solvent did not exceed 1% in any well. Aliquots (0.1 ml) of each antifungal agent at a 2× final concentration were dispensed into the wells of plastic microdilution trays using a Quick Spense II System (Dynatech Laboratories, Chantilly, Va.). The trays were sealed and frozen at −70°C until they were used. The final concentrations of posaconazole were 0.007 to 8 μg/ml.

Media. The agar formulation used for the Etest was RPMI 1640 (American Biorganic, Buffalo, N.Y.) supplemented with 1.5% agar and 2% glucose (RPG agar) and buffered with MOPS. The RPMI 1640 broth medium used for the microdilution testing was buffered with MOPS in accordance with the NCCLS M27-A method (10).

Antifungal susceptibility testing methods. Broth microdilution tests were performed as described in NCCLS document M27-A (10). An inoculum concentra-
results at the lower limit were left unchanged. Discrepancies between MICs of no – 15). Off-scale MICs at twofold level of the reference method for comparison (13 microdilution MICs read at 48 h. Since the Etest scale has a continuous gradient inhibition ellipse was ignored. 1

ment M27-A using Candida species as determined by the reference broth microdilution method. The posaconazole MICs obtained were consistent with values reported previously for the individual Candida spp. tested in RPMI 1640 medium (11, 12). Posaconazole MICs of 1 μg/ml were observed for only three isolates of C. albicans (MICs of >8 μg/ml) and five isolates of C. glabrata (MICs of 2, 4, and >8 [three isolates] μg/ml).

Table 2 summarizes the percentages of 48-h posaconazole MICs obtained by the Etest in RPM agar that were within two dilutions of the reference method result. Overall, the agreement was 95%. The agreement between Etest and microdilution MICs was >90% for C. albicans (98%), C. glabrata (93%), C. tropicalis (97%), and C. krusei (100%). With the exception of C. glabrata, when a discrepancy was observed between the results obtained by the Etest and the reference method, the Etest provided a lower MIC. In the case of C. glabrata, discrepant MICs determined by the Etest were always higher than those determined by the reference method.

The results of this study provide the first documentation of the applicability of the Etest method for determining the in vitro susceptibilities of Candida species to the investigational triazole posaconazole. As in previous studies, we found that RPMI agar with glucose (2% final concentration) supported optimal growth of all species tested and provided excellent agreement with the MICs obtained with the broth microdilution method (Table 2). Similar to the case with the other triazoles, fluconazole (13) and voriconazole (15), the problem of trailing end points due to partial inhibition of growth by azoles was minimized by use of RPMI agar and strict adherence to specific criteria for reading Etest MICs as described in the Etest package insert and technical guide for yeasts (AB BIODISK). Good agreement with broth dilution MICs was observed when discernible growth within an established ellipse was ignored.

In summary, we have provided the first evidence of the ability of the Etest to generate posaconazole MIC data that are comparable to those obtained by the NCCLS microdilution method. RPMI agar with 2% glucose may be used to determine reference quality MICs with the new investigational triazole (voriconazole and posaconazole) and echinocandin (caspofungin) Etest reagents in tests with Candida spp. (15; Pfaller et al., submitted). The availability of Etest reagents for these new antifungal agents will be useful to clinical laboratories because it will provide the flexibility to test one or more of these agents selectively as they are introduced into clinical practice and as the clinical situation dictates.

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