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Proposed Quality Control Parameters for Disk Diffusion Tests with Anidulafungin (2008)

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ABSTRACT

Background: Anidulafungin (ANID) is a new echinocandin with high potency compared to other class agents (micafungin, caspofungin) via a ß-glucan synthesis inhibition mechanism. An 8-laboratory study determined potential disk diffusion (DD) quality control (QC) zone diameter ranges with four Clinical and Laboratory Standards Institute (CLSI)-recommended QC strains.

Methods: QC strains (*C. parapsilosis* [CPAR] ATCC 20019, *C. albicans* [CALB] ATCC 90028, *C. krusei* [CKRU] ATCC 6258, *C. tropicalis* [CTRO] ATCC 750) were tested in eight laboratories using 2 disk lots (MAST #216096 and BD #8018129) and 3 Mueller-Hinton agar lots (Accumedia #101617, Oxoid #612271, BD #7093809) each supplemented with 0.5 µg/ml methylene blue and 2% glucose. Each site tested the QC strains by CLSI M44 method on 10 separate occasions generating 1,920 ANID results overall, along with 1,440 results for 2 control antifungals (fluconazole, voriconazole). Ranges were selected to contain 95% of results while minimizing the breadth to ≤12 mm, where possible. 2-µg ANID disks also contained 1% DMSO + 0.1% polysorbate-80 (Odabasi et al., 2003).

Results: Intra-laboratory zone range variations were 8-19, 10-18, 11-19 and 13-18 mm for the 4 QC strains. Also inter-laboratory median zone variations were extreme with results of 18-24, 26-38, 23-37 and 25-36 mm among QC organisms. Attempts to include 95% of results in range were achieved (Table), but proposed QC ranges were wide (13 to 18 mm). CPAR and possibly CKRU offer narrowest reproducible DD QC ranges.

	Proposed ranges in mm (% in range):	
QC Organism	All Laboratories	Seven Laboratories
CPAR ATCC 22019	15-28 (95.9)	15-27 (95.5)
CALB ATCC 90028	22-41 (95.3)	24-39 (95.0)
CKRU ATCC 6258	20-38 (97.4)	20-35 (94.7)
CTRO ATCC 750	21-39 (96.4)	21-38 (94.6)

Conclusions: ANID DD QC requires wide ranges (13-18 mm) to contain ≥95% of reported results due to extensive intra- and inter-laboratory variation. ANID disk content (DMSO + P-80; used for CPAR testing) may require further refinement.

INTRODUCTION

Anidulafungin is a newer echinocandin antifungal agent with well documented potency against *Candida* spp. with a MIC_{90} value of 0.06 µg/ml reported for a sample of 2,869 *C. albicans* isolates representing a six year collection. As for all echinocandins, anidulafungin MIC_{90} values for *C. parapsilosis* and *C. guilliermondii* are higher (2 µg/ml). Among the three echinocandins studied by Pfaller et al, the rank order of anti-*Candida* potency (MIC_{90} results) was micafungin (0.015 µg/ml) > caspofungin (0.03 µg/ml) > anidulafungin (0.06 µg/ml), although coverage for 99% of isolates was very similar at 1-2 µg/ml. In contrast, Ostrosky-Zeichner et al [2003] recorded essentially equal MIC_{50} results for anidulafungin and micafungin (0.03-0.06 µg/ml) and high values (0.5-1 µg/ml) for caspofungin. The cited potency data were generated for these antifungals using susceptibility test methods developed by the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards [NCCLS]). Recently the disk diffusion method has been adopted for assessing selected azole agents and caspofungin, including quality control (QC) guidelines for the 5-µg disk.

Disk diffusion antifungal susceptibility testing method theoretically offers greater simplicity and may permit wider utility by clinical microbiology laboratories. However, anidulafungin appears to diffuse poorly, perhaps in part due to low aqueous solubility, further complicated by wide differences in potency against target *Candida* spp., e.g. *C. parapsilosis* or *C. guilliermondii* compared to more commonly isolated candidal organisms. Odabasi et al. tested the effects of adding solvents and surfactants to the disks to enhance diffusion (larger zones), a concept further developed by others. The present study utilized commercially-prepared 2-µg anidulafungin disks supplemented with 1% DMSO and 0.1% polysorbate-80 in an attempt to generate QC limits for disk zone diameters against selected CLSI-recommended QC strains.

MATERIALS AND METHODS

The study employed the NCCLS M23-A2 design. Eight laboratories generated replicate zone diameter results using two 2-µg anidulafungin disk lots (MAST lot no. 216096 and BD lot no. 8018129) and three Mueller-Hinton agar powder lots (Accumedia lot no. 101617, Difco lot no. 7093809 and Oxoid lot no. 612271). Four QC organisms (*C. albicans* ATCC 90028, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019 and *C. tropicalis* ATCC 750) were tested daily in each laboratory for 10 days.

The NCCLS M44-A2 disk diffusion method was used with the recommended Mueller-Hinton agar supplemented with 2% glucose and 0.5% methylene-blue (MHA-GMB), added by the commercial supplier. Fluconazole (25-µg) and voriconazole (1-µg) disks were utilized as internal control agents with 97.2% of results observed within published ranges. Zone diameters were measured where "there was a predominant reduction in growth" (approximately 50% inhibition) after 24 hours incubation.

Results were analyzed by methods suggested in NCCLS M23-A2, attempting to minimize breadth of the QC range and to achieve ≥95% of reported results within the proposed QC limits. These ranges were compared to those recently reported for caspofungin. A *C. parapsilosis* ATCC 22019 strain zone diameter of ≥15 mm was considered optimal to discriminate between isolates of this species with high wildtype MIC values from those isolates having mutations of the echinocandin (caspofungin) target site (MIC, ≥4 µg/ml).

An additional collection of 41 strains were selected to challenge the 2-µg anidulafungin disk test for detection of strains having elevated echinocandin MIC values (≥2 µg/ml). Nine *C. guilliermondii* strains having resistant-level caspofungin MIC results (>8 µg/ml) were kindly provided by Professors M.A. Pfaller and D. Diekema (University of Iowa College of Medicine, Iowa City, Iowa); anidulafungin MICs of 2-4 µg/ml. From the 2007 worldwide antifungal surveillance program (SENTRY Program), 25 *C. parapsilosis* (21) and *C. guilliermondii* (4) with seven other strains (four *C. parapsilosis*, three *C. guilliermondii*) each having anidulafungin MIC values at 2 and 4 µg/ml, respectively, were tested using two anidulafungin disk lots.

RESULTS

- Table 1 shows the distributions of reported zone diameters from seven of eight laboratories participating in the QC trial. One laboratory submitted results that significantly differed (larger zones) and those data were eliminated from analysis.
- Extensive intra-laboratory zone range variations were noted for each of four QC strains e.g. 8-19, 10-18, 11-19 and 13-18 mm, but no significant differences in the zones were recognized between the disk lots (median/mean variations of 0-3 mm; average 1.5/1.5 mm) or among the medium lots.

Table 1. Zone diameter distribution for anidulafungin disks in a M23 quality control investigation. Data excludes one laboratory having unacceptable results (Laboratory F); 404-408.

	Occurrence by quality control organism results/strains			
Zone Diameter (mm)	*C. albicans* ATCC 90028	*C. krusei* ATCC 6258	*C. parapsilosis* ATCC 22019	*C. tropicalis* ATCC 750
12			1	
13			0	
14			2	
15			17[a]	
16			21[a]	
17			38[a]	
18			48[a]	
19		5	47[a]	1
20		16[a]	41[a,b]	7
21	2	38[a]	49[a]	11[a]
22	6	31[a]	31[a]	13[a]
23	5	30[a]	17[a]	20[a]
24	11[a]	28[a]	27[a]	21[a]
25	30[a]	45[a]	27[a]	41[a]
26	26[a]	27[a,b]	15[a]	37[a]
27	32[a]	18[a]	8[a]	30[a]
28	40[a]	22[a]	5	39[a,b]
29	33[a]	25[a]	5	31[a]
30	44[a,b]	29[a]	3	31[a]
31	25[a]	20[a]	0	20[a]
32	31[a]	16[a]	1	28[a]
33	20[a]	7[a]	1	15[a]
34	22[a]	14[a]		15[a]
35	18[a]	16[a]		8[a]
36	22[a]	8		13[a]
37	11[a]	4		7[a]
38	10[a]	5		6[a]
39	11[a]	2		11
40	3			0
41	2			2
42	2			
43	1			
No.	408	406	404	407

a. Proposed ranges including 94.6-95.5% of all participant results.
b. Median value in mm.

Table 2. Proposed anidulafungin (2-µg) disk diffusion quality control (QC) ranges.

	Proposed ranges in mm (% in range)	
QC organism	All participants	Seven participants
C. albicans ATCC 90028	22-41 (95.3)	24-39 (95.0)
C. krusei ATCC 6258	20-38 (97.4)	20-35 (94.7)
C. parapsilosis ATCC 22019	15-28 (95.9)	15-27 (95.5)
C. tropicalis ATCC 750	21-39 (96.4)	21-38 (94.6)

Table 3. Use of the 2-µg anidulafungin disk test to categorize strains as susceptible to anidulafungin (≤2 µg/ml) for species having elevated echinocandin MIC values; tentative susceptible zone breakpoint at ≥14 mm.

		Occurrences at anidulafungin zone disk diameter (mm):														
Candida species (no. tested)	Anidulafungin MIC (µg/ml)	6	7	8	9	10	11	12	13	14	15	16	17	18	19	≥20
C. guilliermondii (16)	4	5[a]	-	-	1	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	1	1[b]	-	2	-	1	5[c]
C. parapsilosis (25)	4	2	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	2	-	-	-	-	-	-	-	-	1	1	3	2	1	2	12
All (41)	4	7	-	-	1	-	-	-	-	-	-	-	-	-	-	1
	2	-	-	-	-	-	-	-	-	2	2	3	4	1	3	17

a. Three strains had caspofungin MIC at >8 µg/ml.
b. Caspofungin non-susceptible strain (MIC, >8 µg/ml).
c. All five strains had caspofungin MIC at >8 µg/ml.

- Attempts to utilize the medians calculation to establish an optimal QC zone range produced percentages of zone diameter results within proposed ranges falling below 95%. Therefore, proposed ranges were expanded to achieve the 95% target (Table 2). These ranges were quite wide (13-18 mm), greater than those reported recently for 5-µg caspofungin disks (8-10 mm).
- The QC range for *C. parapsilosis* ATCC 22019 (QC species with the highest anidulafungin MIC) ranged from 15 to 27 mm, a zone of inhibition profile at the lower limit of the optimal, generally reproducible range for a diagnostic DD test.
- To assess the categorical discrimination of the anidulafungin DD test, we tested selected *C. parapsilosis* and *C. guilliermondii* strains (41 overall) that were echinocandin non-susceptible (MIC, ≥4 µg/ml) or were at the upper end of anidulafungin-susceptibility (2 µg/ml). Consensus of duplicate disk tests showed that strains with caspofungin MIC values at (anidulafungin MIC, 2 or 4 µg/ml) produced 2-µg anidulafungin zones varying from 6 to 20 mm, compared to ≥14 mm for anidulafungin-susceptible (MIC, ≤2 µg/ml) organisms.
- A tentative susceptible zone breakpoint of ≥14 mm was applied. Using the data presented here (Table 3), the categorical error was only 2.4% (a very major error, one *C. parapsilosis* strain at 20 mm), and that reported for caspofungin was 22.2% (false-resistant by DD, breakpoint of ≥11 mm) among 18 similar strains. Other more frequently occurring *Candida* spp. isolates (80.0-84.7%) would be expected to produce much larger zones of inhibition at ≥20 mm, thus this DD method can detect strains having target mutations regardless of widely variable zones among QC organisms.

CONCLUSIONS

- Anidulafungin has potent activity against a wide variety of fungal pathogens in vitro and has potential clinical utility with favorable pharmacological profiles.
- Emergence of resistance to echinocandins, in some cases including anidulafungin, could limit the long-term clinical utility of this class, and also necessitates the development of methods that reliably detect strains with higher MIC values (>2 µg/ml). Accurate species identification of these *Candida* spp. having inherently high echinocandin MIC values will also be required.
- Reference MIC tests for *Candida* spp. appear to be robust and the anidulafungin DD test has emerged as a qualified, valuable diagnostic tool having defined QC ranges. A larger population of wildtype and mutant *Candida* spp. clinical isolates should be tested to confirm these preliminary findings, and to validate the tentative anidulafungin breakpoint zone diameter (≥14 mm; 97.6% categorical accuracy).

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