Paper # **C-133**

A Reproducibility Evaluation of MIC Results with New Investigational Antifungals using the Sensititre® YeastOne® Colormetric Antifungal Susceptibility Panel

N.M.Holliday [1*], S.B. Killian [1], C.C. Knapp [1], A. Espinel-Ingroff [2], M.Pfaller [3]

[1] TREK Diagnostic Systems, Inc., Westlake, OH [2] Medical College of Virginia/VCU, Richmond Virginia [3] University of Iowa College of Medicine, Iowa City, Iowa

TREK™ DIAGNOSTIC SYSTEMS, INC.

25760 First Street
Westlake, Ohio 44145
800.871.8909 • www.trekds.com

ABSTRACT

Reproducibility results using new investigational antifungals between three different laboratories, a major performance criterion used for evaluation of any susceptibility test method, was determined using the Sensititre YeastOne Antifungal Panel which incorporates alamarBlue™ as a colometric indicator. MIC's of three new antifungals, Voriconazole, Ravuconazole, and Posaconazole and two comparator antifungals, Amphotericin B and 5-Flucytosine were determined using a series of ten organisms selected to challenge each antifungal assay. Each drug assay was performed in triplicate on three days at each test site. Assays were manually read after being incubated at 35ºC for 24 and 48 hours. Modal MIC response +/- 1 well and +/- 2 well was calculated for each antifungal/organism set for each site, and for combined sites. Modal MIC response +/- 1 well, between sites for all antifungals, agreed at an average of 95% at 24 hours and 95% at 48 hours. At +/-2 wells modal MIC responses averaged at 100% and 99%. Individual site modal responses +/- 1 well averaged 96%, 98%, and 92% at 24 hours, and 95%, 99%, and 90% at 48 hours. Individual site modal responses at +/- 2 wells averaged 100%, 100%, 100% at 24 hours and 100%, 99%, 98%, at 48 hours. MIC determination using the Sensititre YeastOne Antifungal Susceptibility System shows highly reproducible results between all test sites.

INTRODUCTION / OBJECTIVES

- To evaluate MIC reproducibility results with new investigational antifungals using the Sensititre YeastOne Colormetric Antifungal Susceptibility Panel.
- Reproducibility of test results within a laboratory and between laboratories is a major performance criterion used for evaluation of any susceptibility test method.
- Sensititre YeastOne test panels are designed for use in determining quantitative antifungal susceptibilities (MIC) of non-fastidious yeasts.

MATERIALS & METHODS

Materials

Organisms: The testing at 3 sites consisted of the following:

- 10 reproducibility isolates provided by Dr. Michael Pfaller, University of Iowa

Table 1. Reproducibility Isolates Tested

Isolates	Identification	Isolates	Identification
1. C.parapsilosis 1411	A	*6. C.glabrata 1416*	F
2. C.parapsilosis 1434	B	*7. C.glabrata 2304*	G
3. C.krusei 1419	C	*8. C.albicans 1690*	H
4. C.tropicalis 1836	D	*9. C. parapsilosis ATCC 22019*	I
5. C.glabrata 1341	E	*10. C.krusei ATCC 6258*	J

Table 2. Antifungal Agents Tested and their Ranges

Antifungals	Concentration Range Tested (µg/m/l)	MFG
Posaconazole (New)	0.004-8	Schering Plough
Ravuconazole (New)	0.008-16	BMS
Voriconazole (New)	0.008-16	Pfizer
Amphotericin B (Comparator)	0.016-16	Sigma
5-Flucytosine (Comparator)	0.03-64	Sigma

Methods

Each isolate was tested in triplicate on three separate days by three separate sites.

[1] **TREK Diagnostic Systems, Inc., Westlake, OH**
[2] **Medical College of Virginia/VCU, Richmond Virginia**
[3] **University of Iowa College of Medicine, Iowa City, Iowa**

Susceptibility Testing Methods

- Each isolate was tested using a Sensititre YeastOne Colormetric Antifungal Susceptibility Panel. The panels were set-up and tested according to the manufacturer's instructions.
- The NCCLS reference panel was tested according to the M27 standard.
- The MIC for the test organism was determined by observing the lowest antifungal concentration showing inhibition of growth (as evidenced by no color change).

MATERIALS & METHODS con't

Yeast growth in the antifungal solutions was evident as a change in the colormetric growth indicator from blue (negative) to pink (positive). MIC's are interpreted as the lowest concentration of antifungal solutions remaining blue in color. In the case of isolates that exhibited heavy trailing, the MIC was determined as being the first well exhibiting a significant color difference when compared to the positive growth control well.

- Endpoint determinations of the reference plates were performed according to the guidelines published in the NCCLS Document M27-A, reference method for broth dilution antifungal susceptibility testing of yeasts.

Data Analysis

- Frequency distribution of observed MIC results for the three sites were charted out for each drug bug combination, and combined site modal responses were determined at both 24 and 48 hours of incubation (Table 3).
- Agreement statistics of MIC results compared to modal responses were calculated within +/- 1 and 2 well dilutions at both 24 and 48 hours of incubation.

Table 3. Modal MIC Response of Test Organisms 24/48 Hours

Isolates	PZ	RZ	VZ	AB	FC
A	0.12/0.25	0.12/0.5	0.12/0.25	0.5/1	0.06/0.25
B	0.06/0.12	0.03/0.06	0.03/0.06	0.5/1	0.06/0.12
C	0.25/0.5	0.5/1	0.5/1	1/ 2	16/32
D	0.12/0.5	0.25/0.5	0.06/0.25	1/ 2	0.06/0.12
E	1/>8	1 /4	0.25/2	1/ 2	≤0.03/≤0.03
F	0.5/1	0.06/0.5	0.12/0.5	0.5/1	≤0.03/≤0.03
G	1 /2	1/ 2	0.25/1	0.5/1	≤0.03/≤0.03
H	0.5/1	1/ 2	0.5/1	1/ 2	≤0.03/0.06
I	0.12/0.25	0.12/0.5	0.25/0.5	0.5/2	0.06/0.12
J	0.12/0.12	0.12/0.12	0.06/0.12	0.5/1	8/16

RESULTS

Table 4. Overall % Agreement +/- 1 well at 24 and 48 Hours

Incubation Time	Site #1 TREK	Site #2 VCU	Site #3 Iowa	Average
24 Hours	96	98	92	95
48 Hours	95	99	90	95

RESULTS con't

Table 5. Overall % Agreement +/- 2 well at 24 and 48 Hours

Incubation Time	Site #1 TREK	Site #2 VCU	Site #3 Iowa	Average
24 Hours	100	100	100	100
48 Hours	100	99	98	99

Table 6. % Agreement +/- 1 Well for Each Site at 24 and 48 Hours

Antifungals	TREK-24	TREK-48	VCU-24	VCU-48	IOWA-24	IOWA-48	AVG
Posaconazole	100	98	100	99	92	80	97/92
Ravuconazole	88	98	91	97	86	88	88/94
Voriconazole	93	100	100	94	97	86	97/93
Amphotericin-B	100	100	99	100	86	99	95/100
5-Flucytosine	100	100	100	94	100	97	100/97

Table 7. % Agreement +/- 2 Well for Each Site at 24 and 48 Hours

Antifungals	TREK-24	TREK-48	VCU-24	VCU-48	IOWA-24	IOWA-48	AVG
Posaconazole	100	100	100	99	100	96	100/98
Ravuconazole	100	100	99	98	100	97	100/99
Voriconazole	100	100	100	99	100	98	100/99
Amphotericin-B	100	100	100	100	99	100	100/100
5-Flucytosine	100	100	100	98	100	99	100/99

CONCLUSIONS

- This study was designed to evaluate the YeastOne antifungal susceptibility plate's MIC reproducibility with the newer antifungal agents, which is a major performance criteria used in the evaluation of any susceptibility test method. The data presented here indicate this system is highly reproducible within each laboratory. Similarly, it provides highly reproducible results between laboratories.
- Modal MIC response overall (all 3 sites)+/- 1 well at 24 hours was 95% and at 48 hours was also 95%.
- Modal MIC response overall (all 3 sites) +/- 2 wells at 24 hours was 100% and 99% at 48 hours.