COMPARISON OF DISK DIFFUSION (M44-A) ASSAY WITH REFERENCE (M38-A MICRODILUTION) METHOD FOR TESTING MOULDS AGAINST VORICONAZOLE

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Introduction

The CLSI (formerly NCCLS) has developed a reference method (M38-A document) for antifungal susceptibility testing of filamentous fungi (moulds). However, the CLSI broth microdilution method may be cumbersome and time consuming for suitable use in the clinical laboratory. The Subcommittee has also defined one strain of *Paecilomyces variotii* ATCC MYA-3630 as the QC isolate and six other moulds as reference isolates for testing mould pathogens with voriconazole and other agents. Although reference testing conditions are available for yeast disk testing, this simple methodology is not yet available for testing moulds.

Isolates

The set 191 moulds evaluated by each method and disk are listed in Table 1.

In addition, the QC *C. krusei* ATCC 6258 and *P. variotii* ATCC MYA-3630 strains were included each time isolates were tested. Some isolates were provided by M. Rinaldi.

A. Microdilution Method (CLSI M38-A Document)

Microdilution plates were inoculated with the inoculum suspension (approx.104 CFU/ml) by dispensing 100 µl into each well. Trays were incubated at 35ºC for 24 (*Zygomycetes*), 48 (*Aspergillus* spp., *Fusarium* spp., and *P. variotii*, *P. lilacinus*) and 72 h (*Scedosporium* spp.). Some isolates of *Alternaria* spp. and *Bipolaris* spp. required >72 h of incubation and some isolates of *Alternaria* spp. were incubated at 30ºC. The drug concentration range was 0.03-8 mcg/ml.

Purpose

The purpose of this study was to correlate reference voriconazole MICs to zone diameters in mm for 191 mould isolates.

Study Design

The evaluation included: (i) The determination of voriconazole reference MICs for the set of 191 moulds. (ii) The determination of inhibition zone diameters in mm by using two voriconazole disks (1 and 10 mcg) on two agars (Mueller-Hinton agar+2% glucose and 0.5 mcg/ml methylene blue [MGB for all isolates] and non-supplemented Mueller-Hinton agar [MH for selected 46 isolates]). Agar plates were provided by Hardy Diagnostics. (iii) The coefficient correlation (linear regression analysis) of MICs with zone diameters in mm.

B. Modified M44-A Disk Diffusion Method

Each isolate was tested with both 1 and 10 mcg disks (Pfizer) on MGB agar and 46 isolates were also tested on MH agar. Each MGB and MH agar plate was inoculated with the undiluted inoculum suspensions as described in the M44-A document. Plates were examined at 16, 24, 48, 72 h or until sufficient growth allowed measurement of inhibition zone diameters around the disks.

Zone Diameter Measurement

Zone diameters were measured to the nearest whole mm at the point in which there was a prominent reduction of growth. Hyphal elements inside the inhibition zones and light trailing were ignored.

Results

I. Table 1. In vitro susceptibilities of 191 moulds to voriconazole using the CLSI broth microdilution method (M38-A)

Species	No	Range (mcg/ml)	50%	90%
Aspergillus fumigatus	28	0.06-16	0.5	2
A. flavus	18	0.06-1.0	0.5	0.5
A. nidulans	14	0.03-4	0.5	0.5
A. niger	16	0.12-1.0	0.5	1.0
A. terreus	18	0.03-1.0	0.5	0.5
Fusarium moniliforme	6	2-8	2	NA
F. oxysporum	7	2-8	4	NA
F. solani	9	4-16	8	NA
P. lilacinus	9	0.06-1.0	0.25	NA
Alternaria spp.	8	0.5-2	1.0	NA
Bipolaris spicifera	11	0.25-4	1.0	4
Scedosporium apiospermum	11	0.25-2	0.5	1.0
S. prolificans	7	>16	>16	NA
Absidia corymbifera	7	≥16	>16	NA
Mucor circinelloides	3	≥16	NA	NA
M. ramosissimus	3	>16	NA	NA
Rhizopus arrhizus	12	8-16	8	16
R. microsporus var. *rhizopodiformis*	4	8-16	NA	NA

II. Disadvantages of MGB Agar (Figs. 1-4)

A. fumigatus: No growth at 24 h and difficulty in interpreting inhibition zones

A. terreus: Interference with voriconazole activity

P. lilacinus: Poor or no growth and interference with voriconazole antifungal activity

Alternaria spp.: Poor or no growth at 35ºC

Possible solution: MH agar (MH) (Figs. 1-4)

III. Disadvantages of Voriconazole 10 mcg Disk:

Only this disk can be evaluated on the plate due to zone overlapping (large zones). The correlation was slightly better for the 1 mcg disk (R: 0.9438-0.7239 vs. 0.9493-0.8675).

IV. (i). The correlation coefficient (linear regression analysis) suggested that the best incubation times were (Figs. 5-7):

16 h: Most *Zygomycetes*, especially for *R. arrhizus*

24 h: Other *Zygomycetes*, *A. flavus*, and *A. niger*

48 h: Other *Aspergillus* spp., and *Fusarium spp.*

72 h: *Alternaria* spp., *B. spicifera*, *P. lilacinus*, and *Scedosporium* spp.

(ii). The correlation was superior on MH (R: 0.786) than on MGB (R: 0.528) agar.

Fig. 1. *Aspergillus fumigatus* on Two Media

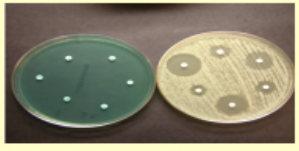


Fig. 2. *Aspergillus terreus* on Two Media

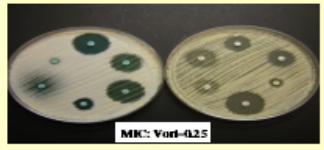


Fig. 3. *Paecilomyces lilacinus* on Two Media

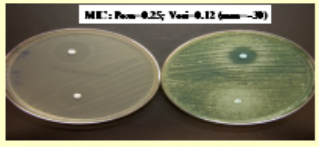


Fig. 4. Two Isolates of *Alternaria* spp. on MH Agar at 30ºC

Fig. 5. Correlation between voriconazole MICs and inhibition zone diameters in mm (1 mcg disk) at 24 h for: *Aspergillus niger*, *A. flavus* and *Zygomycetes*

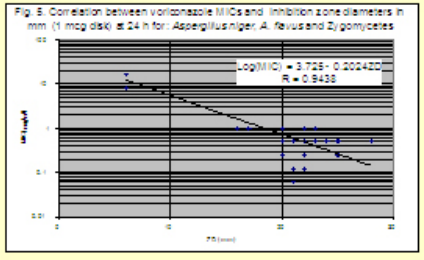


Fig. 6. Correlation between voriconazole MICs and inhibition zone diameters in mm (1 mcg disk) at 48 h for: Other *Aspergillus* spp. and *Fusarium* spp.

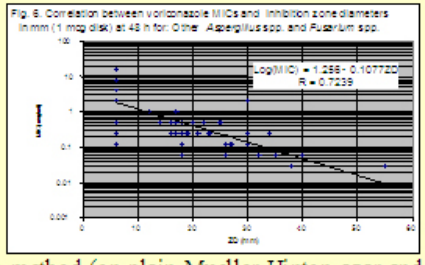
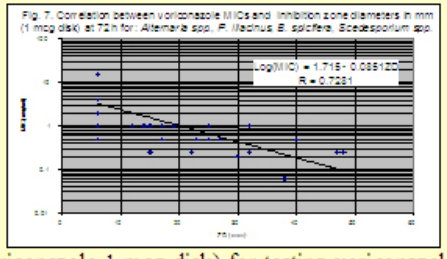


Fig. 7. Correlation between voriconazole MICs and inhibition zone diameters in mm (1 mcg disk) at 72 h for: *Alternaria* spp., *P. lilacinus*, *B. spicifera*, *Scedosporium* spp.



Conclusions: These data suggest the potential value of the simple and more economical disk diffusion method (on plain Mueller-Hinton agar and voriconazole 1 mcg disk) for testing voriconazole against mould isolates in the clinical laboratory.