BIO-RAD

DISKS FOR ANTIFUNGAL SUSCEPTIBILITY TESTING OF YEASTS

CARTRIDGE OF 50 DISKS

STUDY OF THE SUSCEPTIBILITY OF *CANDIDA* SPECIES TO TRIAZOLE ANTIFUNGAL AGENTS (FLUCONAZOLE, VORICONAZOLE)

IVD

1- INTENDED USE

These disks are used to determine the susceptibility of *Candida* species to triazole antifungal agents (fluconazole, voriconazole) by the disk diffusion test. This method follows a standardized procedure published by CLSI/NCCLS(1)

The most recent documents from CLSI/NCCLS should be consulted as guidelines for the procedure to perform the disk diffusion test and its interpretation.

2- PRINCIPLE

Paper disks impregnated with a defined antifungal agent concentration are dispensed onto the surface of the MHGMB (Mueller Hinton agar +2% Glucose and 0.5µg/ml of Methylene Blue) agar inoculated with a calibrated inoculum, prepared from a pure culture of the yeast to be tested. An antifungal concentration gradient is established around the disks dispensed onto the agar. After incubation, the diameter of the zones of inhibition observed around the disks is used to deduce the Minimal Inhibitory Concentration (MIC) of the various antifungal agents (fluconazole, voriconazole) for the tested strain and the interpretive categories: Resistant(R), Susceptible-Dose Dependent(S-DD), Susceptible(S).

3- REAGENTS

The disks, made from high quality absorbent paper, have a diameter of 6.5 mm and are impregnated with antifungal agents at specific concentrations.

These disks are clearly identified by a symbol including 3 letters, followed by the disk content expressed in µg, printed on each side of the disk (Table 1).

The Bio-Rad disks are supplied in cartridges of 50 disks packed in sealed containers containing a desiccant.

Table 1

ANTIFUNGAL AGENT	DISK CONTENT	SYMBOL	PACKAGING	ODERING NUMBER
Fluconazole	25 µg	FCA25	1 x 50 disks	**62802**
Voriconazole	1 µg	VCZ1	1 x 50 disks	**62803**

4- REAGENT STORAGE

The expiration date only applies to disks contained in intact stored cartridges according to the instructions of the manufacturer. The expiration date and the batch number are indicated on each package (cartridge and container).

- The disk cartridges should be stored in their container between + 2 and + 8 ° C **in a dry place**.
- Bring the cartridges to room temperature (18-30°C) before opening .After dispensing the disks, return unused cartridges between + 2 and + 8 ° C .
- Do not use disks after the stated expiration date.
- If the cartridges are stored in the dispenser after use, the dispenser must be stored at + 2-8°C in a dry place **with desiccants** .
- The stability of the disks of opened cartridges placed in the dispensers was validated under routine conditions for 6 weeks.

5- MATERIALS REQUIRED BUT NOT PROVIDED

- disk dispenser: 6 disks-code 50271, 8 disks-code 50270, 12-16 disks-code 50295
- culture medium MHGMB (Mueller Hinton +2% Glucose + 0.5µg/ml of Methylene Blue)
- culture medium: Sabouraud agar(code 56519), Sabouraud Chloramphenicol Gentamicin agar (code 63774)
- inoculum loop
- Pasteur pipettes
- 0.5 McFarland opacity control
- fungal quality control strains
- oven at 35°C +/- 2°C
- laboratory material required for performing antifungal susceptibility testing by the disk diffusion test

6- PRECAUTIONS FOR USERS

Follow the current approved guideline for performing the disk diffusion test (CLSI/NCCLS).

All tests should be conducted using techniques and precautions recommended for protection against microbiological hazards

After use, sterilize the cultures and all the contaminated material.

7- PROCEDURE

a) Culture medium

The M44-A reference method of the CLSI /NCCLS recommends the use of MHGMB agar: Mueller-Hinton agar with 2% Glucose and 0.5µg/ml Methylene Blue.

After preparation of this culture medium as described in the CLSI guideline, pour 25 ml of it into a 90-mm diameter round Petri dish or 60 ml of it in a 120-mm square Petri dish.

After solidification, dry for 15 minutes at 37° before use.

b) Inoculum

Prepare a standardized 0.5 McFarland inoculum from a pure 24- hour-old culture of *Candida* species, obtained from a subculture onto Sabouraud agar or Sabouraud chloramphenicol gentamicin agar.

The inoculum is prepared by picking 5 distinct colonies of approximately 1mm in diameter .Colonies are suspended in sterile saline .

The resulting suspension is vortexed and its turbidity is adjusted by adding sufficient sterile saline or more colonies to adjust the transmittance to that produced by a 0.5 McFarland standard.

c) Inoculation of test plates

Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the suspension. The swab should be rotated several times and pressed firmly against the wall of the tube above the fluid level.

The dried surface of a sterile Mueller-Hinton +GMB agar plate is inoculated by streaking the swab over the entire agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum.As a final step, the rim of the agar is swabbed.

The lid may be left ajar for 3 to 5 minutes, but not more than 15 minutes , to allow for any excess surface moisture to be absorbed before applying the antifungal disks.

d) Application of disks to inoculated agar plates

The antifungal disks are dispensed onto the surface of the inoculated agar plate using a disk dispenser (no more than 6 disks should be placed on a 90-mm round plate, no more than 9 disks on a 120-mm square plate).

e) Incubation

Plates are placed in an incubator set to 35°C +/- 2°C within 15 minutes after the disks are applied.

The incubation period is 24 hours.

f) Reading plates and interpreting results

Measure the diameter of the zones of inhibition after 24 hours of incubation.

The plate is held a few inches above a black, nonreflecting background illuminated with reflecting light.

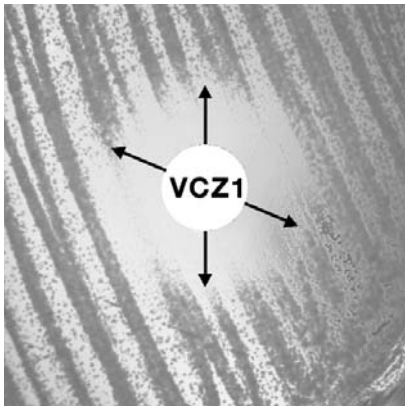
When insufficient growth is observed after 24 hours incubation, re-incubate the plate for an extra 24 hour period and read at 48 hours.

The diameters of the zones of inhibition should be measured up to the colonies of normal size (**Figure1**).

Pinpoint colonies at the zone edge or large colonies within a zone are encountered frequently and should be ignored.

These colonies do not correspond to resistant mutants.

Figure1



g) Notes

- Samples: the antifungal disks should not be used for performing a disk diffusion test directly on biological samples.
- Good Laboratory Practices should be applied at all times.

8- INTERPRETATION OF DISK DIFFUSION TEST RESULTS

- Accurately measure the diameter of the zone of inhibition.
- The correspondence between the diameter of the zone of inhibition, the Minimal Inhibitory Concentration (MIC) and the interpretive categories: Resistant(R), Susceptible-Dose Dependent (S-DD), Susceptible (S) is summarized in the following table :

Code	Antifungal Agent	Disk content	Symbol	Diameter of the zone of inhibition (mm)*			Equivalent MIC Breakpoints(µg/ml)*		
				R	S-DD	S	R	S-DD	S
62802	**Fluconazole**	25 µg	FCA 25	≤ 14	15–18	≥ 19	≥ 64	16–32	≤ 8
62803	**Voriconazole**	1 µg	VCZ 1	≤ 13	14–16	≥ 17	≥ 4	2	≤ 1

*Fluconazole: CLSI/NCCLS guidelines M44-A(1)
Voriconazole: Artemis Disk Global Antifungal Surveillance Study (3)

9- PERFORMANCE / QUALITY CONTROL OF THE TEST

The following table shows the recommended quality control zone diameter(mm) ranges obtained by the disk diffusion test for the quality control strains mentioned hereafter (1) :

Code	Antifungal Agent	Disk content	Symbol	*C. albicans* ATCC 90028	*C. parapsilosis* ATCC 22019	*C. tropicalis* ATCC 750	*C. krusei* ATCC 6258
62802	**Fluconazole**	25 µg	FCA 25	28–39	22–33	26–37	–
62803	**Voriconazole**	1 µg	VCZ 1	31–42	28–37	–	16–25

10-QUALITY CONTROL OF THE MANUFACTURER

All products manufactured and marketed by Bio-Rad are submitted to a Quality Assurance system from reception of the raw materials until marketing of finished products. Each batch of finished product is submitted to quality control tests and is only released onto the market when it complies with all acceptance criteria. Records concerning production and control tests on each batch are kept by the manufacturer.

11-LIMITATIONS OF THE PROCEDURE

The performance of the test depends not only on the activity of the disks, but also on other factors such as the use of a standardized inoculum, of appropriate quality control strains, suitable pre-tested culture media and on an adequate storage temperature for the disks.

12-REFERENCES

1. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts ; Approved Guideline, M44-A, Vol. 24 N°15, NCCLS, May 2004.
2. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts ; Approved Standard-Second Edition, M27-A2, Vol. 22 N°.15, NCCLS, August 2002.
3. **M.A. Pfaller, D.J. Diekema, M.G. Rinaldi, R. Barnes, B. Hu, A.V. Veselov, N. Tiraboshi, E. Nagy, D.L. Gibbs**, and the Global Antifungal Surveillance Group. 2005. Results from the ARTEMIS DISK Global Antifungal Surveillance Study : a 6.5- Year Analysis of Susceptibilities of Candida and Other Yeast Species to Fluconazole and Voriconazole by Standardized Disk Diffusion Testing. J. Clin. Microbiol. **43** : p. 5848-5859.
4. **M.A. Pfaller, D.J. Diekema, J.H. Rex, A. Espinel-Ingroff, E.M. Johnson, D. Andes, V. Chaturvedi, M.A Ghannoum, F.C. Odds, M.G. Rinaldi, D.J. Sheehan, P. Troke, T.J. Walsh, and D.W. Warnock**. 2006. Correlation of MIC with Outcome for *Candida* Species Tested against Voriconazole ; Analysis and Proposal for Interpretive Breakpoints. J.Clin. Microbiol. **44** : p. 819-826.

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