DISKS FOR ANTIFUNGAL SUSCEPTIBILITY TESTING

CARTRIDGE OF 50 DISKS

STUDY OF THE SUSCEPTIBILITY OF FUNGI TO ANTIFUNGAL AGENTS

IVD

1- INTENDED USE

These disks are used to determine the susceptibility of yeasts (*Candida, Cryptococcus*, etc.) and spore-bearing filamentous fungi (*Aspergillus*) to antifungal agents, by an agar diffusion method.

2- PRINCIPLE

Paper disks impregnated with a defined concentration of antifungal agent are deposited on the surface of semi-synthetic medium (5-Fluorocytosin) or casitone medium (Amphotericin B, Nystatin, Econazole, Clotrimazole, Miconazole, Ketoconazole) previously inoculated with a calibrated inoculum prepared from a pure culture of the fungal strain to be tested. An antifungal concentration gradient is established around the disks placed on the agar. After incubation, the diameter of the zones of inhibition observed around the disks is used to deduce the Minimum Inhibitory Concentrations (MIC) of the various antifungal agents for the strain tested and clinical classification (Sensitive, Intermediate, Resistant).

3- HOW SUPPLIED

The 6.5 mm diameter disks, made from superior quality absorbent paper, are impregnated with precise concentrations of antifungal agents.
The disks are clearly identified by a code, comprising 2 or 3 letters, printed on each side of the disk (Table 1).
Bio-Rad disks are supplied in cartridges of 50 disks packaged in watertight containers containing a desiccant.

	DISK DOSE	SYMBOL	PACKAGING	PRODUCT CODE
5-Fluorocytosine	1µg	5FC 1	1 x 50 disks	62846
Amphotericin B	100 µg	AB 100	1 x 50 disks	62806
Nystatin	100 IU	NY 100	1 x 50 disks	62856
Econazole	50 µg	EC 50	1 x 50 disks	62826
Clotrimazole	50 µg	CTR 50	1 x 50 disks	62816
Miconazole	50 µg	MCZ 50	1 x 50 disks	62836
Ketoconazole	50 µg	KET 50	1 x 50 disks	62866

4- STORAGE

The expiry date applies exclusively to disks contained in intact cartridges stored according to the manufacturer's instructions.
The expiry date and batch number are indicated on each packaging (cartridge and container).

- Cartridges of disks must be stored in their containers at a temperature between +2°C and +8°C **in a dry place**.
- Cartridges must be allowed to adjust to room temperature (18-30°C) before opening. After applying the disks, return unused cartridges to a temperature between +2°C and +8°C.
- Do not use disks after the expiry date.
- If cartridges are stored in the dispenser after use, the dispenser must be stored at +2-8°C in a dry place **with desiccant**.
- The stability of disks and opened cartridges placed in dispensers has been validated under routine conditions of use for 6 weeks.

5- MATERIAL REQUIRED BUT NOT PROVIDED

- disk dispenser: 6 disks ref # 50271
  8 disks ref # 50270
  12-16 disks ref # 50295
- culture media (Casitone agar code 55813, Semi-Synthetic medium code 55812)
- sterile distilled water (9 ml tubes code 54154)
- inoculation loop
- Pasteur pipettes
- fungal strains for quality control
- laboratory equipment necessary for antifungal susceptibility testing by the agar diffusion method.

6- PRECAUTIONS FOR USE

Always observe the current techniques and precautions concerning protection against microbiological hazards.
After use, sterilize the cultures and all contaminated material.

7- INSTRUCTIONS

- Samples: Disks must not be used for tests performed directly on biological samples.
- Refer to the instructions for Casitone agar (code 55813) or Semi-Synthetic medium (code 55812) for preparation of the inoculum from a pure, fresh culture, the mode of inoculation, and the incubation temperature and time.
- Good Laboratory Practices should also be applied at all times.

8- INTERPRETATION OF THE RESULTS

- Precisely measure the diameters of the zones of inhibition observed.
- The correspondence between diameter, Minimum Inhibitory Concentration (M.I.C.) and clinical category (Sensitive, Intermediate, Resistant) is summarized in the following table:

	Diameter of the zone of inhibition (mm)	**MIC (µg/ml)**	**Interpretation for yeasts (a – b)**
5-Fluorocytosine* (1 µg)	= 20 20 – 10 = 10	= 1.56 1.56 – 25 = 25	Sensitive Intermediate Resistant
Amphotericin B	< 10 = 10	< 1 = 1	Sensitive Intermediate or Resistant
Nystatin	> 10 = 10		Sensitive Resistant
Imidazoles ** (Econazole, Clotrimazole, Miconazole, Ketoconazole)	= 20 20 – 10 = 10	= 1.56 1.56 – 6.4 = 6.4	Sensitive Intermediate Resistant
5-Fluorocytosine (1 µg)	> 10 = 10		**for *A. fumigatus*** Sensitive Intermediate or Resistant

For strains considered to be resistant (diameter of the zone of inhibition less than 10 mm), determination of the M.I.C. should be performed or the strains should be sent to a mycology reference laboratory.

* For 5-fluorocytosine, the presence of small colonies in the zone of inhibition corresponds to resistant mutants.
** For imidazoles, the evaluation is sometimes difficult, as some strains present two zones of inhibition:

- a large zone
- a smaller zone with small colonies which do not appear to be resistant when susceptibility testing is repeated: growth of these small colonies is also observed during determination of the M.I.C.

a- Results obtained with *Candida albicans* and other yeast-like fungi (1), (3)
b- The Sensitive, Intermediate or Resistant classification is based on comparison of the M.I.C. and serum antifungal concentrations obtained with usual oral or intravenous dosages (2).

Antifungal susceptibility testing of spore-bearing filamentous fungi (such as *Aspergillus fumigatus*) can be performed under similar conditions to those of yeast-like fungi (inoculum = 10_6 spores/ml).

9- PERFORMANCES/QUALITY CONTROL OF THE TEST

The following table presents the acceptable limits for the diameters of the zone of inhibition obtained by agar diffusion for the reference strains indicated below:

	Disk strength	**Acceptable limits of the diameter of the zone of inhibition (mm)**	
		Candida albicans DP 3153A	*Candida albicans* DP 41R5FC
5-Fluorocytosine	1 µg	30 - 38	resistant
Nystatin	100 IU	16 - 25	17 - 26
Econazole	50 µg	23 - 33	25 - 35
Clotrimazole	50 µg	18 - 28	18 - 30
Miconazole	50 µg	18 - 25	20 - 30
Ketoconazole	50 µg	25 - 36	29 - 39
		Candida albicans ATCC 24433	*Candida albicans* ATCC 90028
Amphotericin B	100 µg	15 - 21	15 - 21

10- QUALITY CONTROL OF THE MANUFACTURER

All products manufactured and marketed by Bio-Rad are submitted to a Quality Assurance system from reception of 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- LIMITS OF USE

Test performances depend not only on the activity of the disks, but also on other factors such as the use of a standardized inoculum, appropriate reference strains, suitable pre-tested culture media and an adequate disk storage temperature.

12- REFERENCES

1. DROUHET E., DUPONT B. Bull. Soc. Fr. Mycol. Med. 1978, 2, 165-170 – Antibiogramme des champignons aux antifongiques.
2. DROUHET E., DUPONT B. Encyclopédie médico-chirurgicale (Paris. 1976, 9. La. Infect. 8004 M10: Traitements antifongiques.
3. DROUHET E. et al., Standardization of the antifungal sensitivity tests. Report of the Study Group of the French Society for Medical Mycology, Bull. Soc. Fr. Mycol. Med., 1981, 10, n°1, 131-134.
4. GRILLOT R. Les antifongiques. Présentation synoptique et tests in vitro. Grillot R. (ed). Les mycoses humaines: démarche diagnostique. Elsevier, Paris, 1996, pp. 323-353.

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