

# Fungal Antibiogram

## DEFINITION

Fungal Antibigrams is used for for determination of susceptibility to antifungals.

The method of diffusion in Agar Medium, i.e.:

- solid media distributed in Petri dishes (round or square),
- disks impregnated with different antifungal drugs corresponding to the pharmaceutical specialities available to the clinic.

## PRESENTATION

### Media used

See corresponding Technical Sheet(s)

### Disks impregnated with antifungals

Single cartridges of 30 disks

- **5 fluorocytosine** code 356-2871  
10 µg (Ancotil<sup>®</sup>)
- **5 fluorocytosine** code 356-2841  
1 µg (Ancotil<sup>®</sup>)
- **Nystatin** code 356-2851  
(Mycostatin<sup>®</sup> 100)
- **Amphotéricin B** code 356-2801  
100 µg (Fungizone<sup>®</sup>)
- **Econazole** code 356-2821  
50 µg (Pévaryl<sup>®</sup>)
- **Clotrimazole** code 356-2811  
50 µg (Canesten, Trimysten<sup>®</sup>)
- **Miconazole** code 356-2831  
50 µg (Daktarin<sup>®</sup>)
- **Ketoconazole** code 356-2861  
50 µg (Nizoral<sup>®</sup>)

## INTERPRETATION

Antifungals	Ø of zone of inhibition (in mm)	C.M.I. in µg/ml	Interpretation (a-b) for yeasts
5 fluorocytosine* (1 µg)	≥ 20 20-10 ≤ 10	≤ 1.56 1.56-25 ≥ 25	Sensitive Intermediate Resistant
Amphotéricin 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 1.56-25 ≥ 25	Sensitive Intermediate Resistant
5 fluorocytosine 1 µg	> 10 ≤ 10	> 10 ≤ 10	For <i>A. fumigatus</i> Sensitive Intermediate or resistant
5 fluorocytosine 10 µg	> 10 ≤ 10	> 10 ≤ 10	Sensitive or Intermediate Resistant

For strains considered resistant (Ø of zone of inhibition < 10 mm), it is recommended that MIC be determined or that the reference strain be sent to:

Pr. DROUHET  
Institut Pasteur  
25 rue du Docteur Roux  
75015 PARIS - FRANCE

\* Colonies found in the 5-fluorocytosine zone of inhibition are among the resistant strains.

\*\* Evaluation is sometimes difficult for imidazole compounds. Certain strains present 2 zones of inhibition:

- a large zone,
- a smaller zone with small colonies that do not appear resistant when their sensitivity is tested

# Fungal Antibiogram

again: growth of these small colonies is also observed during determination of MIC

a) Results obtained with *Candida albicans* and other yeast like fungi.

b) Strains that are sensitive, intermediate or resistant compared to serum levels obtained with usual doses by oral or intravenous route.

The volume of media to be dispensed is 15 ml\*, corresponding to:

- 1 round	} Thickness of agar: 2.4 mm
Ø 90 mm dish	
- 3 round	
Ø 50 mm dish	

Dishes filled with agar should be dried for 30 minutes at 37°C before use.

\* Volume contained in a presentation = 1 tube

## METHODOLOGY

### Inoculum

#### • For *Candida* and *Torulopsis*

The contents of a calibrated inoculating loop, loaded with a 24-hour culture of yeasts, is homogenized in 10 ml of distilled water so as to obtain a suspension of approximately 10<sup>6</sup> yeasts cells per ml. A 1/10 dilution of this suspension (1 ml, i.e. 20 drops of a Pasteur pipette of suspension in 9 ml of sterile distilled water), i.e. 10<sup>5</sup> yeasts cells per ml, serves as inoculum.

#### • For *Cryptococcus neoformans*

The inoculum is prepared from a calibrated inoculating loop loaded with spore-forming culture of about 5 days for 10 ml of distilled water.

#### • For *Aspergillus fumigatus*

The inoculum is prepared from a calibrated inoculating loop loaded with spore-forming culture about 5 days for 10 ml of distilled water. The spore-forming culture is obtained on Sabouraud + Chloramphenicol agar at 37°C (green culture).

As a result of the "inoculum" effect for certain antifungals (5FC and imidazoles), the aim is to obtain confluent colonies at the moment of reading.

### Inoculation

- Flood the whole surface of the agar with:
  - 5 ml of inoculum for Ø 90 mm dish
  - 2 ml for Ø 50 mm dish any excess suspension being drawn off with a pipette
- Dry the dishes for 15 minutes at 37°C

NB: Using tweezers, deposit the disks on the agar.

### Application of disks

Take care to respect the relationship established between media and antifungals according to the indications provided at the beginning of the document (media used).

### Pre-diffusion and incubation

In order to obtain pre-diffusion of antifungals, it is best to leave the dishes for 30 minutes at room temperature before placing them in the incubator at 37°C.

- for 24 hours for *Candida*, *Torulopsis* and *Aspergillus*,
- for 48 hours for *Cryptococcus neoformans*.

### Reading and interpretation

For each antifungal:

Measure the diameter of the zone of inhibition – using a caliper rule or compasses - as close as possible to the surface of the agar.

### STORAGE

- Media: see corresponding Technical Sheet(s)
- Disks: + 2-8°C in a dry place
- Expiration date and batch number are shown on the package

### QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

### BIBLIOGRAPHY

• REGLI P., FERRARI H., GOUDARD M., BUFFARD Y. (1982): Intérêt du milieu Casitone pour l'étude de la sensibilité, *in vitro*, des champignons levuriformes aux antifongiques dérivés de l'imidazole. Bulletin de la Société Française de Mycologie Médicale, **11**: 359-362

• DROUHET E. et col. (1981): Standardisation de l'antibiogramme antifongique. Rapport du groupe d'étude de la Société Française de Mycologie Médicale, Bulletin de la société Française de Mycologie Médicale, **10**: 131-134

• DROUHET E., DUPONT B. (1978): Antibiogramme des champignons aux antifongiques. Bulletin de la Société Française de Mycologie Médicale, **2**: 165-170

# Fungal Antibiogram

- **DROUHET E., DUPONT B. (1976):** Traitements antifongiques. Encyclopédie médico-chirurgicale, **9**: maladies infectieuses, 8004<sup>10</sup>