



ANTIMICROBIAL SUSCEPTIBILITY
SYSTEMS

Technical Information

Mic6 (Hexa) Antimyco-01

Product Code: HX1104

Application: Hexa Antimyco-01 is an inert flat circular ring having 6 discs of 6 mm diameter on its projections. These discs are coated with antibiotics that aid antibiotic susceptibility testing of fungal cultures.

Composition**

Each ring contains

Antibiotic	Concentration
Amphotericin-B (AP)	100Unit
Clotrimazole (CC)	10µg
Fluconazole (FLC)	25µg
Itraconazole (IT)	10µg
Ketoconazole (KT)	10µg
Nystatin (NS)	100Unit

Susceptibility Test Procedure:

1. Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (DM1063) and incubated at $35 \pm 2^\circ\text{C}$. Colonies are suspended in 5ml of sterile 0.85% Saline.
2. Vortex the resulting suspension and adjust the turbidity to yield $1 \times 10^6 - 5 \times 10^6$ cells /ml (i.e. 0.5 McFarland standard).

Test Procedure:

1. Prepare plates with Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye (DM2825) for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the petri plate) and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
3. Apply the discs using aseptic technique. Deposit the discs at the center of the plate.
4. Invert the plates and place in an incubator set to $35 \pm 2^\circ\text{C}$ within 15 minutes after the discs are applied.
5. Examine each plate after 20 - 24 hours of incubation. If plate was satisfactorily streaked the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. Read at 48 hours only when insufficient growth is observed after 24 hours incubation.
6. Record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW1096/PW297)



Principle

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2).

For convenience and economy of conducting antimicrobial susceptibility tests multidiscs are designed. These are enhanced extensions of Single Discs. These series of discs gives the privilege to study large number of antibiotics at one time.

These discs are made of unique inert material which enhances their absorption hence allowing faster adhesion of discs to the media. Moreover the discs are designed in such a way that each antibiotic on a single ring is at least 24 mm apart from the others, thus reducing the merging of zones. The symbols and concentrations of antimicrobials present are indicated in respect of each peripherally located disc.

Quality Control

Appearance

Flat circular ring of inert material w/ 6 equidistant arms on the outer periphery, each arm having a 6 mm disc at the end; each disc impregnated w/ different antibiotics, w/ corresponding symbols & concentrations printed on the ring.

Cultural response

Average diameter of zone of inhibition observed on Mueller Hinton Agar (DM1173) + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye Medium after 18 -48 hours of incubation at 35-37°C for standard cultures.

Organisms(ATCC)	Antibiotic	Std.Zone of diameter(mm)
<i>Candida albicans</i> ATCC 10231	Amphotericin -B AP 100 units	10 -18 mm
	Clotrimazole CC 10 mcg	12 -18 mm
	Fluconazole FLC 25 mcg	25 -30 mm
	Itraconazole IT 10 mcg	18 -22 mm
	Ketoconazole KT 10 mcg	18 -22 mm
	Nystatin NS 100 units	15 -23 mm
<i>Candida albicans</i> ATCC 90028	Amphotericin -B AP 100 units	10 -17 mm
	Clotrimazole CC 10 mcg	18 -32 mm
	Fluconazole FLC 25 mcg	28 -39 mm
	Itraconazole IT 10 mcg	16 -20 mm
	Ketoconazole KT 10 mcg	20 -32 mm
	Nystatin NS 100 units	19 -27 mm
<i>Candida parapsilosis</i> ATCC 22019	Amphotericin -B AP100 units	11 -20 mm
	Clotrimazole CC 10 mcg	16 -30 mm
	Fluconazole FLC 25 mcg	22 -33 mm
	Itraconazole IT 10 mcg	11 -18 mm
	Ketoconazole KT 10 mcg	14 -29 mm
	Nystatin NS 100 units	16 -25 mm
<i>Candida tropicalis</i> ATCC 750	Amphotericin -B AP100 units	8 -12 mm
	Clotrimazole CC 10 mcg	10 -20 mm
	Fluconazole FLC 25 mcg	26 -37 mm
	Itraconazole IT 10 mcg	8 -13 mm
	Ketoconazole KT 10 mcg	17 -28 mm
	Nystatin NS 100 units	16 -21 mm
<i>Candida krusei</i> ATCC 6528	Amphotericin -B AP100 units	9 -14 mm
	Clotrimazole CC 10 mcg	14 -24 mm
	Itraconazole IT 10 mcg	8 -15 mm
	Ketoconazole KT 10 mcg	10 -14 mm
	Nystatin NS 100 units	15 -20 mm



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<i>Sacchromyces cerevisiae</i> ATCC 9763	Amphotericin -B AP100 units	11 -18 mm
	Clotrimazole CC 10 mcg	17 -25 mm
	Nystatin NS 100 units	17 -25 mm

Storage and Shelf Life

Discs should be stored between 2 to 8°C under dry conditions, along with the dessicator provided in individual pack. For prolong use, store below -20°C. Use before expiry date on the label.

Disclaimer

- User must ensure suitability of the product(s) in their application prior to use.
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