



ANTIMICROBIAL SUSCEPTIBILITY
SYSTEMS

Technical Information

Clotrimazole CC 10 mcg

Product Code: SD1115

Application: Clotrimazole CC 10 mcg discs are used for antimicrobial susceptibility testing of fungal cultures

Composition**

*Ingredients	Concentration
Clotrimazole	10 mcg/disc

Susceptibility Test Procedure:

Preparation of Inoculum:

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 1×10^6 - 5×10^6 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 with centers at least 24 mm apart.
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.

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 Kirby- Bauer 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 any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.



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Quality Control

Appearance: Filter paper discs of 6mm diameter with printed "CC 10" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye after 24-48 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)*
<i>C.albicans</i> (90028)*	18-32
<i>C.parapsilosis</i> (22019)*	16-30
<i>C.tropicalis</i> (750)*	10-20
<i>C.krusei</i> (6528)*	14-24
<i>C.albicans</i> (10231)	12-18
<i>S.cerevesiae</i> (9763)	17-25

*= Q.C. Strains recommended by CLSI

Further Reading

1. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guidelines-Third edition Vol.38 No.24, Dec- 2018 CLSI document M44-A2. For more details refer to this volume
2. Zone Diameter Interpretive Standards, Corresponding Minimal Inhibitory Concentration (MIC) Interpretive Breakpoints, and Quality Control Limits for Antifungal Disc Diffusion Susceptibility Testing of Yeasts, Third Informational Supplement CLSI Document- M44- S3- Aug 2009.

Storage and Shelf Life

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

Disclaimer

- User must ensure suitability of the product(s) in their application prior to use.
- The product conforms solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate.
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