



Amphotericin-B AP (Part A: 32-0.25 µg & Part B: 0.256 -0.002 µg)

MD071

Amphotericin-B HiComb™ MIC Strip is a rapid and reliable method for determining the antimicrobial susceptibility of different microorganisms against Amphotericin-B. This system provides a set of 16 different concentrations in gradient that can be easily used to deduce a functionally accurate the Minimum Inhibitory Concentration (MIC) in microgram levels.

Composition

Part Levels in µg of Amphotericin-B

Part A: 32, 16, 8, 4, 2, 1, 0.5, 0.25

Part B: 0.256, 0.128, 0.064, 0.032, 0.016, 0.008, 0.004, 0.002

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 (M063) 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 (M1825) 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 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 strips using aseptic technique as follows
 - i. Pick up one strip by its handle with a sterile forceps
 - ii. Place the strip with its higher concentration facing the edge of plate and the markings on strip facing up wards.
 - iii. Press gently on the handle of the strip and assure that all the discs are in full contact with the medium.
 - iv. Place other strip on the opposite side of plate with higher concentration towards the edge of plate and lower concentration towards the centre.
 - v. Close the plate and invert to check whether all the discs are in full contact with the medium [6 Strips (3 of A and 3 of B) can be placed on a 200 mm plate with the markings facing upward]
4. 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.
5. The zone of inhibition will be in the form of an ellipse. MIC value would be the value at which the zone convenes the comb-like projections of the strips and not at the handle. If there is no zone of inhibition observed, report the MIC as greater than the highest concentration on the strip. If the zone of inhibition is below the lowest concentration then report the MIC as less than the lowest concentration.

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. Minimum Inhibitory Concentration (MIC) determination is a Quantitative method. Determining MIC using a conventional broth dilution method is a tedious and time consuming process.

Hence Gradient diffusion susceptibility testing method is accepted widely and is in routine use for a long time. HiComb is also based on innovative gradient-based technique. This system is developed using dry chemistry technology and consists of two comb shaped strips made of an inert material with 8 extensions that carry the discs of 4 mm, resembling the 'tooth' of a comb. Towards the stem of the strip, MIC reading scale in µg/ml is given along with the HiMedia code. A defined concentration of antibiotic is loaded on each of the disc so as to form a gradient when placed on agar plate. HiComb based on the principle of dilution and diffusion, consists of a gradient that cover a continuous range across 16 two-fold dilutions as per the conventional MIC method. When applied to the agar surface, the antibiotic instantaneously diffuses into the surrounding medium in high to low amounts from one end of the strip to the other. The gradient remains stable after diffusion, and the zone of inhibition created takes the form of ellipse. The MIC value would be the value at which the zone convenes the comb-like projections of the strips and not at the handle. The MIC value obtained from this system can be compared to the standardized CLSI procedure (2).

HiComb is advantageous for routine use as it is simple to set up and perform, and is rapid than any agar or broth dilution method. The test has special advantage to study the resistance surveillance. The wide concentration gradients of these tests cover the MIC ranges of susceptibility of a wide variety of pathogens and allow both low level and high-level resistance detection.

Quality Control:

Appearance: Comb like inert strips having 16 different concentrations of Amphotericin-B (8 on each strip) impregnated on the circular filter paper discs having diameter of 4mm with the concentrations printed on the strips.

Cultural response: Zone of inhibition in form of ellipse is observed on Mueller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye (M1825) after incubation at 35-37°C for 24-48 hours for standard cultures.

Organisms (ATCC)	Std. MIC range (µg)
<i>C.albicans</i> (90028)	0.125-0.5
<i>C.parapsilosis</i> (22019)	0.25-1
<i>C.krusei</i> (6528)	0.5-2
<i>C.tropicalis</i> (750)	0.064-0.25
<i>C.albicans</i> (10231)	0.016-0.25

Storage and Shelf-life:

Strips 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.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guidelines-Second edition Vol.29 No.17, August- 2009 CLSI document M44-A2. For more details refer to this volume

Note:

1. This strip is INTENDED ONLY FOR AGAR DIFFUSION METHOD AND NOT FOR BROTH DILUTION METHOD.
2. HiComb should be used for *in vitro* diagnostic purpose only and aseptic procedures and precautions should be used when handling bacterial samples throughout the testing procedure.
3. Before using HiComb strips, ensure that the strip is at Room temperature or 25°C.
4. Pick the HiComb strip by its handle.

5. If accidentally dropped on to a dry surface, pick the strip up and use it.
6. If dropped in, an incorrect position, leave the strip there and use the other region of the plate.
7. When applying strips be steady. Do not move the strip once in contact with the agar surface, since the antibiotic instantaneously diffuse on contact with agar.
8. For fastidious organisms or very susceptible species, use only 4 to 5 strips on a 150 mm plate and one on a 90 mm plate.

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