Amphotericin-B AP 20 mcg

Intended Use

Amphotericin-B AP 20 mcg discs are used for antimicrobial susceptibility testing of fungal cultures.

Principle

Antimicrobial Susceptibility Testing (AST) is a laboratory procedure performed by laboratory technician to identify, which antimicrobial regimen is specifically effective for individual patients. The introduction of various antimicrobials for treating variety of infections showed the necessity of performing antimicrobial susceptibility testing as a routine procedure in all microbiology laboratories. antibiotics are generally defined as the substances produced by the microorganism such as Penicillium, which has the ability to kill or inhibit the growth of other microorganisms, mainly bacteria. Antimicrobial Susceptibility Tests (ASTs) basically measures the ability of an antibiotic or other antimicrobial agent to inhibit the *In vitro* microbial growth.

The basic principle of the antibiotic susceptibility testing has been used in microbiology laboratories over 80 years. Till the 1950s, laboratories were lacking in the methodologies and equipment's for the accurate determination of *In vitro* responses of organisms to antimicrobial agents. Bauer *et al.,* began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. Antimicrobial Susceptibility Tests are either quantitative or qualitative.

Clinical laboratories currently employ several methods depending on the laboratory test menu that they provide. These approaches include the disk diffusion and Minimum Inhibitory Concentration (MIC) methods. 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.

Various regulatory agencies and standards-writing organizations, published standardized reference procedures 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) for any antimicrobial testing, Quality control or clinical testing. However, few precautions are to be maintained while handling of the Sensitivity discs, the latest CLSI documents should be consulted for current recommendations.

Susceptibility Test Procedure

Fungal Inoculum Preparation

1. Aseptically take a loopfull of culture from subcultured slant or broth and transfer it to the SDA plate for primary, secondary & tertiary streaking.
2. Incubate the freshly inoculated plate for 18-24 hours at 35°C-37°C.
3. Select 3-4 similar colonies of approximately 1mm and transfer them into 5mL of sterile 0.85% saline.
4. Vortex the resulting suspension and adjust the turbidity to yield a uniform suspension matching Mc Farland standard (O.D at 620nm range: 0.08-0.130)
5. Prepare plates with Mueller Hinton Agar (201130650100/201130650500) +2% Glucose and 0.5 µg/mL Methylene Blue Dye (GMB) Medium for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm.
6. Inoculate the entire agar surface of the plate three times, rotating the plate 60° between streaking to obtain even inoculation. Swab the rim of the agar bed too.
7. The lid may be left ajar for 3-5 minutes and the plate held at room temperature for not more than 15 minutes to allow the surface moisture to be absorbed before applying the antibiotic discs.
8. Apply discs by means of an antimicrobial disc dispenser, aseptically, at least 24 mm apart.
9. Within 15 minutes of applying the discs, invert the plates and incubate at 35°C-37°C.
10. Examine plates after 24-48 hours of incubation. The diameters of the zones of complete inhibition are measured, as determined by gross visual inspection.

Zones are measured to the nearest whole millimeter. For further details in measuring zones of inhibition, consult the reference. If only isolated colonies grow, the inoculum is too light and the test should be repeated. Zones around discs containing different drugs are not comparable for the purpose of comparing activity of drugs. See the Zone Diameter Interpretive Chart, which gives expected values from testing common aerobes.

Quality Control

Appearance: Filter paper disc of 6 mm diameter with printed ''AP 20'' on each side of the disc.
Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar with 2% glucose and 0.5 mcg/ml methylene blue dye after 20- 24 hours of incubation at 35°C-37°C for standard cultures.

Organism (ATCC)	Standard Diameter of zone of inhibition in mm
Candida albicans 3147 (10231)	10-16
Saccharomyces cerevisiae NRRL Y-567 (9763)	8-12

Storage and Shelf-life:

Discs in routine use should be stored at 2°C-8°C. Longer term storage should be at -20°C-8°C.

References:

1. 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
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.
3. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
206010600250	Antimicrobial Susceptibility Disc	5 Carts (5 x 50 disc)
206010600500	Antimicrobial Susceptibility Disc	5 Vials (5 x 100 disc)
206010600100	Antimicrobial Susceptibility Disc	Single Vial (1 x 100 Disc)