

# Amphotericin B 20 U

Cat. 7087

6-mm discs prepared by impregnating high quality absorbent paper with accurately determined amounts of antibiotic or other chemotherapeutic agents.

## Practical information

Industry: Clinical / Antimicrobial susceptibility testing



## Principles and uses

An antibiogram allows to in vitro subject a standard bacterial inoculum to one or different antibiotic concentrations and, after analyzing results, predict in vivo effectiveness. The interpretation of results (Sensitive, Intermediate or Resistant) is carried out on the basis of parameters previously established by a number of committees, such as The Clinical and Laboratory Standards Institute in the U.S., or The European Committee on Antimicrobial Susceptibility Testing in Europe. The standardized procedures are based on the Bauer-Kirby method.

## Formula in g/L

Amphotericin B

20

## Instructions for use

» For clinical diagnosis, the type of samples are pure cultures.

1.- MEDIUM: the standard culture medium for the production of the antibiogram is Mueller Hinton (Cat. 1058) and Mueller Hinton II (Cat. 1055) , intended for the growth of many aerobic organisms. For fastidious organisms, such as streptococci and gonococci, 5% defibrinated blood must be added to Mueller Hinton and Muller Hinton II. For Haemophilus influenzae, Haemophilus Test Medium (Cat. 1434) must be used. Ready-to-use plates must be prepared following the data sheet instructions for each medium.

2.- PREPARATION OF MATERIAL: 4-5 colonies that show similar morphology are taken from the primary isolation medium with a flame loop, and suspended in 4-5 ml broth: Mueller Hinton Broth (Cat. 1214), Trypticasein Soy Broth (T.S.B.) (Eur. Pharma)(Cat.1224) or Brain Heart Infusion Broth (Cat. 1400). They are incubated at 37°C for 2-5 hours. If there is turbidity at the end of this time, adjust the turbidity with a McFarland Standard Tube 0.5 (equivalent to a uniform suspension of 1.5x10<sup>8</sup> bacterial cells/ml). If the bacteria concentration is low, continue incubating for 3 more hours and adjust.

3.- INOCULATION INTO PETRI DISH: prepared bacterial suspension is homogeneously mixed and streaked, turning the plate into the Mueller Hinton Petri Dish (or other appropriate agar), with the help of a sterile cotton swab. Another technique is to pour several ml into the agar, distribute homogeneously and remove excess fluid. After inoculation, leave the plates unsealed for 10-15 minutes to allow for any surface moisture to be absorbed before applying the drug- impregnated discs.

4.- ANTIBIOTIC DISC APPLICATION: Allow containers to reach room temperature before opening. Open the CONDA Antibiotic discs cartridge under the flame and discharge the disc with the trigger or flame and cooled forceps. Press the disc gently into the agar with the help of the forceps. The space between the discs must not be narrower than 24 mm, and the distance to the edge no less than 1 cm. CONDA Special Dispenser 6x and 8x may also be used. It is preferable to

deposit penicillin and cephalosporin discs no less than 10 mm from the edge of the Petri dish, with their centers at least 30 mm apart. With H. influenzae, N. gonorrhoeae and S. pneumoniae, use no more than nine discs per 150 mm plate, or four discs per 100 mm plate.

5.- INCUBATION: Place the Petri Dish into the incubator inversely at 37°C for 18-24 hrs. For Staphylococcus spp., testing at temperatures above 35°C may not detect methicillin resistant staphylococci (MRS); for N. gonorrhoeae, incubate at 36 ± 1°C. Haemophilus spp., N. gonorrhoeae, S. pneumoniae and other streptococci should be incubated in an atmosphere enriched with 5% CO<sub>2</sub>.

6.- RESULTS: The diameter of the inhibition zone is read from the underside of the disc. If Mueller Hinton with Blood has been used, inhibition areas must be read from the surface with a compass or ruler. Zones are measured to the nearest whole millimeter. The inhibition areas must be evaluated according to the Table provided by CONDA.

Depending on the results, the bacteria strains will be evaluated as SUSCEPTIBLE (S), INTERMEDIATE (I) and RESISTANT (R).

SUSCEPTIBLE (S): this category implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the recommended dosage is used on the infected site.

INTERMEDIATE (I): This category includes antimicrobial isolates whose MICs that are close to blood and tissue levels, and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated (e.g. quinolones and  $\beta$ -lactams in urine), or when a higher than normal dosage of a drug can be used.

RESISTANT (R): this category implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules, and/or that demonstrate zone diameters that fall within the range where specific microbial resistant mechanisms (e.g.  $\beta$ -lactams) are likely, and clinical efficacy of the agent against the isolate has not reliably been shown in treatment studies.

#### POSSIBLE SOURCES OF ERROR:

- 1.- Culture a suspension that has not been carefully adjusted against 0.5 standard tube. If the test has been carried out on broth with too high bacterial concentration, narrow zones can be obtained.
- 2.- Use of mixed culture. An essential principal of the standardized culture method is the use of pure culture. A mixed culture can cause faulty results.
- 3.- Presence of excess wet on the medium surface can cause excess growth and this can make the inhibition zone smaller. Contrarily, a very dry surface can cause poor growth and a larger inhibition zone.
- 4.- If the discs form incorrect zones with the recommended control organisms, the entire procedure should be checked. A faulty zone size maybe due to the disc, the inoculation, the preparation or depth (about 4 mm) of the medium, or other factors.

#### FURTHER INFORMATION:

The latest CLSI documents should be consulted for current recommendations.

Undertake precautions against microbiological hazards throughout all procedures. Sterilize cultures, containers and other contaminated materials after use.

The classifications of Resistant, Intermediate and Susceptible vary only by one millimeter, which is within normal laboratory error. Some cultures may give a borderline zone that varies from day to day or from laboratory to laboratory; such cultures are relatively uncommon.

## Microbiological test

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The product mentioned above has been approved according to the accepted procedures that are prescribed and validated thoroughly by the main microbiological circles.

The information mentioned above is accurate to the best of our knowledge and belief. However, neither the product nor the information is provided with warranty for any specific use, apart from the CLSI and EUCAST criteria and the condalab prospectus.

## Storage

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Temp. Min.: -20 °C

Temp. Max.: 8 °C

## Bibliography

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1. Clinical Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement. CLSI document M100-S19. Wayne, PA: CLSI; 2009.
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3. Ericsson, H.M., and J.C. Sherris. 1971. Antibiotic Sensitivity Testing, Report Study, Acta Pathol, Microbiol. Scand., Section B, Suppl. 217, 1-90.
4. European Committee on Antimicrobial Susceptibility Testing (EUCAST): Expert rules in antimicrobial susceptibility testing, 2008. Disponible en: <http://www.srga.org/eucastwt/MICTAB/index.html>
5. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: general principles and contemporary practices. Clin Infect Dis. 1998;26:973-80.