biolab

TECHNICAL SHEET

IVD CE

BIOLAB
ANTIMICROBIAL SUSCEPTIBILITY
TESTING DISCS

INTENTED USE:

Disc diffusion is one of the oldest and most commonly used methods in routine clinical laboratories for antimicrobial susceptibility testing. It is suitable for testing; bacteria, fastidious bacterias and also including common bacterias. Discs containing antifungal agent are examined by disc diffusion method. Bacitracin, optochin, nitrocefin, PYR, Oxidase etc. discs are not used for therapeutic purpose in determining the susceptibility and resistance of bacteria; used for differentiation of bacterial strains.

EXPLANATION:

Bauer and Kirby1 applied this agar disc diffusion method by absorbing antimicrobial agents into absorbent paper. Various disc diffusion QC limits (mm) are given by CLSI 2-3, EUCAST4 institutions. In clinical bacteriology, bacterial sensitivity against Antibiotics, Sulphamids and Antifungi has been usually detected by using agar diffusion technique. Biolab® AST discs have been carefully standardized by using special absorbant paper which have the ability of high liquid absorption capacity. Biolab disc papers have a diameter of 6 mm and are coded with the code of antimicrobial. Each cartridge contains 50 discs.

STANDARDIZED SENSITIVITY TEST:

PREPARATION OF MEDIUM: It is generally accepted that, Mueller-Hinton medium is the most suitable one for this purpose. For this reason, at quality control assay in final inspection, this medium has been used. When sterilized, medium comes to 45-50 °C, and then it is poured into Petri dishes. The agar depth must be 4 mm ± 0.5 mm. For 90 mm diameter plates, 25 ml of medium is enough. For 100 mm diameter plates, 31 ml of medium; for 150 mm diameter plates 71ml medium and for 100 mm square plates 40 ml medium is enough. pH of the medium must be 7.2 to 7.4. Medium petri dishes should be stored at between +4 °C and +8 °C. If plates are stored for longer than a week, storing must be in sealed bags in order to prevent the evaporation and drying. To remove excess wetness and droplets on the surface of plates, it is necessary to keep them in incubator for 30 minutes or one hour at room temperature before using. The plates must not be dried. This medium without adding blood is adequate for growth of many aerobic organisms. But for fastidious organisms such as streptococci and gonococci, %5 defibrinated sheep or horse blood must be added after cooling the medium up to 45-48°C. Plates can be used when their surface is wet and the results obtained by this method are reliable. Before autoclaving; 2% Glucose and 0.5 mcg / mL Methylene Blue Dye (GMB) should be added to Mueller Hinton Agar, which will be used in the study of antifungal discs.

STORAGE:

On receipt, store discs at -20 to +8°C. The expiry date is valid only for unopened blister packs stored under proper conditions. Once a cartridge is open, it is recommended that it is stored for no more than 7 days. Allow containers to come room temperature before opening in order to minimize condensation as this may reduce the potency of the antimicrobial agent. Once opened, discs should be stored within the dispenser in the container provided or other suitable opaque air tight desiccated container to protect discs from moisture. Containers should be stored within the dispenser in the refrigerator and be allowed to come to room temperature before opening to prevent the formation of condensation. Return unused discs to the refrigerator when application of discs has been completed. Use the oldest disc first. Discard expired discs.

PREPARATION OF INOCULUM:

From primary isolation medium, 4-5 colonies that show similar morphology are either taken by direct colony suspension method and by using a flamed loop they are being suspended or by the help of a sterile cotton swab the bacteria are picked up and suspended in 4-5 ml sterile saline solution (solution of %0,9 NaCl in water). Direct colony suspension method can be used for fastidious organisms too. If a visible turbidity is obtained at the end of this time, the turbidity of bacterial suspension must be adjusted against 0.5 McFarland Standard Tube (preparation of McFarland 0.5 turbidity standard: add 0.5 ml of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2.2H_2O$ to and 99.5 mL of 0.18 mol/L (0.36 N) H_2SO_4 (%1 v/v) and mix thoroughly). It is appropriate to do this adjustment on a paper with black strips on it. Standard tubes must be vortexed before usage (except for the tubes that does not require vortex). The density of suspension is adjusted with a spectrophotometer. At 625 nm absorbance, the turbidity must be adjusted between 0.08 - 0.13. After preparation of standard suspension, it must be used within an hour. Once every six months standard McFarland tubes must be renewed.

The denser suspension causes the inhibition zone to shrink, while the less dense suspension causes the zone to become larger.

INOCULATION INTO PETRI DISHES:

It has been done according to the technique reported by Bauer and Kirby. Prepared bacterial suspension is mixed with a sterile applicator and excess fluid of applicator is removed by slightly pressing and rotating the applicator inside the tube, above the fluid level. Streak the entire agar surface of a Mueller Hinton Agar (or other appropriate agar) plate three times, turning the plate 60° between streakings to obtain even inoculation. After this, allow Petri dishes to dry for 3-5 minutes (max. 15 mins) at room temperature (this is done for preventing the excessive wetness of the medium).

Firstly, cartridge is opened under flame and then discs are discharged from cartridge into the Petri dish by means of the forceps or dispenser. It is necessary to press gently into the dispersed discs by the help of a flamed and cooled forceps. The discs have to be put into the petri dishes within 15 minutes. To see inhibition zone clearly, the space between discs must not be narrower than 24 mm and the distance of discs from the edge of the plate must be 12 mm. The discs should be removed from the refrigerator 30 minutes before use and be allowed to come to room temperature before using.

Within 30 minutes, Petri dishes must be put into incubator inverse position. After incubation at 35±1°C for one night (16-20 hours), measure the diameters of zones of inhibition to the nearest milimetre with a ruler.

READING INHIBITION ZONES:

After overnight incubation (16-20 hours) clear inhibition zones are obtained in antibiogram plates, prepared in suitable manner. At the end of 6-8 hours primary inhibition zone can be seen, but this must be confirmed by overnight zone. The diameter of inhibition zone is read from the underside of the dish. If the zone will be calculated in blood agar, the inhibition zone must be measured by mm in the surface of the medium. Interpretation (for clinical and also quality control criteria) of zones has been found from EUCAST/CLSI. Accordingly, clinical results are obtained either evaluated as susceptible (S), intermediate (I) or resistant (R) for clinical application.

SPECIMENS: If possible, specimens should be taken from patients before antimicrobial therapy is initiated.

POSSIBLE SOURCES OF ERROR:

- Culture suspension that has been carefully adjusted against standard tube: If the test has been done from broth that bacteria concentration is low, broad zones can be obtained. If concentration is heavy, narrow zones can be obtained.
- Using of mixed culture: Essential principal of standardized culture method is the usage of pure culture. Mixed culture can cause faulty results.

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QUALITY CONTROL STRAINS:

CLSI

a. Nonfastidious:	Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 35218, Klebsiella pneumoniae ATCC 700603.
b. Fastidious:	Haemophilus influenzae ATCC 49247, Haemophilus influenzae ATCC 49766, Neisseria gonorrhoeae ATCC 49226, Streptococcus pneumoniae ATCC 49619.

EUCAST

a. Nonfastidious:	Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 29213, Escherichia coli ATCC 35218.
b. Fastidious:	Haemophilus influenzae ATCC 49247, Haemophilus influenzae ATCC 49766, Neisseria gonorrhoeae ATCC 49226, Streptococcus pneumoniae ATCC 49619.

REFERENCES:

1- Bauer, A.W., W.M.M. Kirby, J.C. Sherris, anda M.Turck. 1966. Am. J. Clin. Pathol. 45:493-496

2- CLSI. Performans Standarts for Antimicrobial Susceptibility Testing. 29th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standars Institute;2019.

3- CLSI. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. 3rd ed. CLSI guideline M45. Wayne, PA: Clinical and Labotatory

4- EUCAST disk diffusion method for antimicrobial susceptibility testing. Version.3,0 April 2013

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