ANTIMICROBIAL SUSCEPTIBILITY SYSTEMS

Technical Information

*Mic6 (Hexa) G-plus 25

Product Code: HX1047

Application: Hexa G-plus 25 is an inert flat circular ring having six discs of 6 mm diameter on its projections. These discs are coated with antibiotics that aid antibiotic susceptibility testing of Gram Positive Organisms

Composition**

Each ring contains

Antibiotic	Concentration
Ceftriaxone (CTR)	30µg
Chloramphenicol (C)	30µg
Clindamycin (CD)	2µg
Erythromycin (E)	15µg
Levofloxacin (LE)	5µg
Tetracycline (TE)	30µg

Susceptibility Test Procedure:

1.Prepare plates with Mueller Hinton Agar (DM1173/DM2084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. For Haemophilus spps use Haemophilus Test Agar(DM2259+MS2117), for S.pneumoniae Muller Hinton Agar supplemented with 5% Sheep Blood is to be used, & for Neisseria spps : G.C.Agar +1% defined growth supplement(DM1434+MS2025)is recommended. The medium in the plates should be sterile and should have a depth of about 4 mm.

2.Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (DM1011) and incubate at 35-37°C for 2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (R092) (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08-0.13 OD turbid suspension at 625 nm)

3.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.

4.Apply the discs using aseptic technique.

5.Deposit the rings at the centre of the plate using sterile forceps

6.Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.

7.Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW1096/PW1297)

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 Bauer-Kirby 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 convenience and economy of conducting antimicrobial susceptibility tests multidiscs are designed. These are enhanced extensions of Single Discs. These series of discs gives the privilege to study large number of antibiotics at one time.

These discs are made of unique inert material which enhances their absorption hence allowing faster adheration of discs to the media. Moreover the discs are designed in such a way that each antibiotic on a single ring is at least 24 mm apart from the others, thus reducing the merging of zones. The symbols and concentrations of antimicrobials present are indicated in respect of each peripherally located disc.

Quality Control

Appearance

Flat circular ring of inert material w/ 6 equidistant arms on the outer periphery, each arm having a 6 mm disc at the end; each disc impregnated w/ different antibiotics, w/ corresponding symbols & concentrations printed on the ring.

Cultural response

Average diameter of zone of inhibition is observed on Mueller Hinton Agar (DM1173) after 18-24 hours incubation at 35-37°C for standard cultures.

Organisms(ATCC)	Antibiotic	Std.Zone of diameter(mm)
Escherichia coli ATCC 25922	Ceftriaxone CTR 30 mcg	29 -35 mm
	Chloramphenicol C 30 mcg	21 -27 mm
	Levofloxacin LE 5 mcg	29 -37 mm
	Tetracycline TE 30 mcg	18 -25 mm
Staphylococcus aureus ATCC 25923	Ceftriaxone CTR 30mcg	25 -31 mm
	Chloramphenicol C 30 mcg	19 -26 mm
	Clindamycin CD 2 mcg	24 -30 mm
	Erythromycin E 15 mcg	22 -30 mm
	Levofloxacin LE 5 mcg	19 -26 mm
	Tetracycline TE 30 mcg	24 -30 mm
Pseudomonas aeruginosa ATCC 27853	Ceftriaxone CTR 30 mcg	17 -23 mm
	Levofloxacin LE 5 mcg	19 -26 mm

Further Reading

1.Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2.Performance standards of Antimicrobial Disc Susceptibility Tests, CLSI Vol. 32 No.3, Jan 2012.

Storage and Shelf Life

On receipt discs should always be stored at -20°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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