



ANTIMICROBIAL SUSCEPTIBILITY
SYSTEMS

Technical Information

Co-Trimoxazole COT 25 mcg (Trimethoprim/Sulphamethoxazole) (1.25/23.75 mcg)

Product Code: SD1010

Application: Co-Trimoxazole (Trimethoprim/Sulphamethoxazole) COT 25 mcg (1.25/23.75mcg) discs are used for antimicrobial susceptibility testing of bacterial cultures as per Kirby-Bauer Method

Composition**

*Ingredients	Concentration
Co-Trimoxazole (Trimethoprim/Sulphamethoxazole)	25 mcg/disc (1.25/23.75 mcg)

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (DM1173/DM2084) for rapidly growing aerobic organisms as per Kirby- Bauer Method. 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 (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. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
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 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)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.



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Interpretation:

Use following interpretive criteria for susceptibility categorization*

Antimicrobial agent	Interpretative criteria for	Sensitive	Intermediate	Resistant
		mm or more	mm	mm or less
Co-Trimoxazole (Trimethoprim/ Sulphamethoxazole) 25 mcg (1.25/23.75 mcg)	<i>Enterobacteriaceae</i> , <i>Acinetobacter</i> , <i>B.cepacia</i> , <i>S.maltophilia</i> , <i>Staphylococcus</i> , <i>Haemophilus</i> <i>influenzae</i> & <i>Haemophilus parainfluenzae</i>	16	11-15	10
	<i>Neisseria meningitidis</i> .	30	26-29	25
	<i>S.pneumoniae</i>	19	16-18	15

Quality Control

Appearance: Filter paper discs of 6mm diameter with printed "COT 25 (1.25/23.75mcg)" on centre of each side of the disc.

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

Organisms (ATCC)	Std. zone of diameter (mm)*
<i>E.coli</i> (25922)	23-29
<i>S.aureus</i> (25923)	24-32
<i>E.faecalis</i> (29212)	>=20
<i>S.aureus</i> (29213)	26-32
<i>E.faecalis</i> (29212)	26-34 (as per EUCAST standards)

Further Reading

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 29th Ed., CLSI Vol. 39 No.1, Jan 2019.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 9.0, valid from 01.01.2019.

Note:

1. Use following media to carry out susceptibility test
2. For rapidly growing aerobic organisms : Mueller Hinton Agar (DM1173/DM2084)
3. For *Haemophilus* spps : Haemophilus Test Agar (DM2259 + MS2117)
4. For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
5. For *Neisseria* spps : G.C.Agar +1% defined growth supplement (DM1434 + MS2025)

* Not for Medicinal Use

Storage and Shelf Life

Discs 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.

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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