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

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

REF: BS.1/ST01.050.0050	50 Discs	REF: BS.1/ST25.050.1250	1250 Discs
REF: BS.1/ST05.050.0250	250 Discs		

METHOD 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 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.

Use following media to carry out susceptibility test For rapidly growing aerobic organisms :
Mueller Hinton Agar For Haemophilus spps : Haemophilus Test Agar For S.pneumoniae : Muller Hinton Agar supplemented with 5% Sheep Blood For Neisseria spps : G.C.Agar +1% defined growth supplement.

Composition

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

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the Co-Trimoxazole (Trimethoprim/Sulphamethoxazole) COT 25 mcg (1.25/23.75mcg) material safety data sheet.

STORAGE AND STABILITY

BioScien Co-Trimoxazole (Trimethoprim/Sulphamethoxazole) COT 25 mcg (1.25/23.75mcg) should be stored between -20 to 8 °C in a firmly closed container. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar for rapidly growing aerobic organisms as per Bauer-Kirby 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 and incubate at 35-37°C for2-8 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

EQUIPMENTS REQUIRED NOT PROVIDED

- Sterile Petri dishes
- Sterile test tubes
- Sterile cotton swap
- Incubator
- Autoclave

QUALITY CONTROL

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

Microorganisms	Std. zone of diameter (mm)*
E.coli (25922)	23-29
S.aureus (25923)	24-32
E.faecalis (29212)	>=20
S.pneumoniae (49619)	20-28

* = Interpretive criteria & QC ranges as per CLSI standards.

REFERENCES

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

SYMBOLS IN PRODUCT LABELLING			
IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number	⚠	Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		