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EN Oxoid Antimicrobial Susceptibility Test Discs

INTENDED USE: Oxoid Antimicrobial Susceptibility Test Discs are used in the semi quantitative agar diffusion test method for in vitro susceptibility testing. **Summary and Explanation:** A suitable agent for use in vivo use can then be determined using filter paper discs impregnated with specified concentrations of antimicrobial agents placed on the surface of a suitable test medium. **Principles of the Procedure:** Pure cultures of the clinical isolates are inoculated onto the test media. The antibiotic diffuses through the agar to form a gradient. After incubation the zones of inhibition around the discs are measured and compared against recognized zone size ranges for the specific antimicrobial agents under test. **Reagents:** Oxoid Antimicrobial Susceptibility Test Discs are 6mm filter paper discs bearing an alpha-numeric code identifying the antimicrobial agent and concentration, printed on both sides. Oxoid discs are supplied in cartridges of 50 discs. There are 5 cartridges in each pack. Cartridges are individually packed in a foil-sealed blister pack with a desiccant. **Precautions:** For in vitro diagnostic use only. Follow directions for use. Observe aseptic techniques and established precautions against all microbiological hazards throughout all procedures. Cultures, containers and other contaminated materials must be sterilized after use in accordance with guidelines for the handling and disposal of biohazardous waste. Do not use the product beyond the stated expiry date. Once the cartridge is open ensure it is stored in an opaque desiccated environment to prevent degradation of the antibiotic. If the discs do not produce the expected zone sizes with recommended control organisms, check the entire procedure. **Storage Instructions:** Unopened cartridges must be stored at -20°C to 8°C until required. Unopened cartridges should be allowed to come to room temperature before removing them from the packaging to minimize condensation as this may reduce the potency of the antimicrobial agent. The expiry date is valid only for unopened blister packs stored under proper conditions. Once the cartridges are open they need to be stored within the dispenser in the container provided (please ensure the desiccant is charged), or other suitable opaque air tight container with a charged desiccant to protect the discs from moisture. Dispensers should be stored within the container in the refrigerator and be allowed to come to room temperature before opening to prevent the formation of condensation. Once a cartridge is open, it is recommended that it is stored for no more than 7 days. **Specimens:** Microorganisms under test should be fresh, pure clinical isolates from culture media. If possible, specimens should be taken from patients before antimicrobial therapy is initiated.

PROCEDURE:

Materials provided: Oxoid Antimicrobial Susceptibility Test Discs. **Materials required but not supplied:** Agar plates with appropriate media, inoculum suspension medium, sterile loops and swabs, sterile forceps, McFarland turbidity standards, incubator, modified atmosphere environments, antibiotic disc dispensers, quality control strains, apparatus to measure zone sizes and interpretative criteria for local standard methods. **Method:** Oxoid discs may be used with a variety of standard testing methodologies including, but not restricted to the BSAC, CDS, DIN, CLSI M2 or EUCAST please refer to current local guidelines for specific information relating to recommended media, inoculum level and incubation conditions. **Quality Control Procedures:** It is recommended that control strains are tested at appropriate intervals; this can either be when each test is performed, or as recommended in the guidance from the national reference groups for antibiotic susceptibility testing. If the result obtained for a control strain organism against a specific antibiotic is outside the range specified, the patient results should not be reported and the discs should not be used until the reason for the discrepancy is determined (e.g. media, fill volume, inoculum, incubation conditions, control strain or incorrect storage or application of the disc).

Limitations: This product is for in vitro diagnostic use only to give an indication of the in vivo susceptibility of the test organism. The selection of antimicrobial agents to test and report is a decision that must be made by each clinical laboratory. The decision to use an antibiotic for therapy against the test organism is the responsibility of the clinician who will take into account other factors which may influence the in vivo activity of the compound.

Summary of the Clinical Laboratory Standards Institute (CLSI) method (other standards will be different): **Media:** Mueller-Hinton agar is used to test non-fastidious organisms, Mueller-Hinton with 5% sheep blood to test *N. meningitidis* and *Streptococcus* spp. GC agar with 1% defined growth supplement to test *N. gonorrhoeae* and Haemophilus Test medium for *H. influenzae* and *H. parainfluenzae*. Media and test conditions for other fastidious or rarely tested bacteria can be found in the CLSI M45. Allow plates and discs to equilibrate to room temperature before use. Plates should not have excess moisture before inoculation.

Test Organisms: Implement appropriate test methods to verify presumptive identification of the test organism. A standardized inoculum is prepared using either the direct colony or growth method and used to inoculate the plates within 15 minutes of preparation: Growth method – Touch 3–5 isolated morphologically similar colonies with a loop, and transfer to 4–5mL of Tryptic Soya Broth or similar. Incubate at 35°C for 2–6 hours. Adjust the turbidity with sterile saline or broth to achieve a turbidity equivalent to a 0.5 McFarland standard (to prepare a 0.5 McFarland standard add 0.5mL of 0.048M Barium chloride (1.175% wt/vol $BaCl_2.2H_2O$) to 99.5mL of 0.18mol/L sulphuric acid (1% vol/vol) – keep this standard in the dark). Direct Colony method (preferred method for staphylococci and streptococci) – Take isolated colonies from a non selective agar into either sterile saline or broth and prepare a suspension equivalent to a 0.5 McFarland standard. **Inoculation**: Immerse a sterile swab in the suspension and rotate against the side of the tube to remove the excess fluid. Swab the entire surface of the plate in at least three directions. Discs should be applied to the plate within 15 minutes of inoculation. **Discs:** Allows discs to come to room temperature before use. The recommended number of discs tested per plate is shown below:

Organism:	Recommended discs per plate:
N.gonorrhoeae, *H. parainfluenzae*, *H. influenzae*, and *Streptococcus* spp.	9 discs on 140–150mm plate or 4 discs on 90–100mm plate
N. meningitidis	5 discs on 140–150mm plate or 2 discs on 90–100mm plate
Non-fastidious organisms	12 discs on 140–150mm plate or 5 discs on 90–100mm plate

Invert the plate and incubate as indicated below within 15 minutes of disc application

Organism:	Incubation Requirements:
H. influenzae and *H. parainfluenzae* 5%	CO_2, 35±2°C, 16–18 hours
N. gonorrhoeae	5% CO_2, 36±1°C, 20–24 hours
N. meningitidis	5% CO_2, 35±2°C, 20–24 hours
Non-fastidious organisms*	Aerobic, 35±2°C, 16–18 hours
Streptococcus spp.,	5% CO_2, 35±2°C, 20–24 hours

*Staphylococci and enterococci require 24 hours incubation. Methicillin-resistant staphylococci may not be detected at temperatures above 35°C.

Interpretation: After incubation measure the diameters of the zones of complete inhibition to the nearest mm. Zone margins should be read as the area showing no obvious growth detected by the unaided eye. Blood plates should be read using the upper surface of the plate with reflected light (do not read the edge of haemolysis, but the edge of growth). All other media should be read from the back of the plate using a black surface and reflected light (except staphylococci and enterococci which are read with transmitted light – faint in-growth is indicative of resistance). With *Proteus* spp. ignore the thin veil of swarming in-growth in an obvious zone of inhibition. For full instructions relating to the interpretation of the results according to the CLSI methodology please refer to the current CLSI M100 standard. A table showing the CLSI compound/concentrations can be found on the web site. Other compounds and concentrations are available from Oxoid for use with other standard methods, but are not detailed.

References:

1. Clinical Laboratory Standards Institute (CLSI) 2009. M2-A9. Performance Standards for Antimicrobial Disk Susceptibility Tests.
2. Clinical Laboratory Standards Institute (CLSI) 2010. M100-S20. Performance Standards for Antimicrobial Disk Susceptibility Tests. Informal Supplement
3. Clinical Laboratory Standards Institute (CLSI) 2006. M45-A. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently isolated or Fastidious Bacteria.

Glossary of Symbols:

IVD For in vitro diagnostic use
Consult instructions for use
Expiry date
CE Comformite Europeene
LOT Batch number of the product
Manufacturer
Storage temperature
Period after opening

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