ASTD
ANTIMICROBIAL SUSCEPTIBILITY TEST DISCS

IVD

Spiramycin 100
Cat. no: E110113

PACKAGE SIZE
1 x 50 discs (1 cartridge) packed into capped tube including desiccant or blister pack
5 x 50 discs (5 cartridges) packed into capped tube including desiccant

INTRODUCTION
Antimicrobial susceptibility tests discs are used in microbiological diagnostics to categorise microorganisms into susceptible, intermediate and resistant groups. The information is very useful in antimicrobial therapy. The discs should be used according to an appropriate standardised method. These standards are based on original methodology by Bauer-Kirby [1] and EUCAST recommendations. The method published by the Clinical Laboratory Standards Institute (CLSI) is probably the most widely recognised.

PRINCIPLE OF METHOD.
Antibiotic impregnated paper discs are placed onto the surface of a sufficient agar plate, which has been inoculated with the test organism. During incubation the antibiotic diffuses from the disc to the medium. Susceptibility of the test organism is visualised as a circular zone of growth inhibition around the disc [1-5].
The zones of inhibition are measured and compared with standard data.

KIT CONTENT
The 6mm of diameter discs, made from high quality paper. The discs are impregnated with accurately assayed quantities of antibiotic or other chemotherapeutic agent. Discs are clearly printed on both sides with letters and numbers designating the agent and drug content.

MATERIALS REQUIRED BUT NOT PROVIDED
Culture media (available in BioMaxima)
Reference strains (available in BioMaxima)
Laboratory equipment necessary to perform the tests (spectrophotometer)

STORAGE
1. Store the discs in original containers, at temperature specified on the label. (Normally at 2-8°C, some discs (e.g. β-lactams) should preferably be kept frozen at -20°C).
2. The unopened disc containers should be removed from the refrigerator or freezer 1 or 2 hours before use, so they may equilibrate to room temperature before opening. This procedure minimizes the amount of condensation that occurs when warm air contacts cold discs. Unused discs should be returned to the refrigerator promptly.
3. If the vials are stored in the fridge which is opened frequently (what causes temperature liability), there should be kept only the number of vials possible to use within a week.
4. The expiry date concerns only the discs stored as directed. Do not use the discs after expiry date.
5. In case of using the discs from 1 vial several times within 7-14 days, the remaining discs shall be checked with reference strains. If the results are positive, the discs can be admitted for further use then.
6. In case of receiving wrong growth inhibition zones during the testing with reference strains, the susceptibility testing procedure must be verified. Incorrect results may be caused by wrong conditions of storage, the quality of medium or procedure mistakes.

STABILITY
The antimicrobial susceptibility discs are stable up to the expiry date when stored in closed containers according the manufacturer recommendations mentioned on the label.

PROCEDURE
1. Prepare the bacterial suspension (inoculum) of the turbidity of 0,5 McFarland, as per EUCAST recommendations.
2. Inoculation. Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab dip into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside walls of the tube above the fluid level. This will remove excess inoculum from the swab. Then inoculate the prepared suspension over the whole surface of sufficient agar plate (Table 1). The agar surface should be swabbed in 3 different directions by turning the plate at 60° angle between each streaking. Allow the inoculated plates to dry (no more than 15 minutes) leaving the lid on.
3. Apply disks (no more than 15 minutes after inoculation of plates) using aseptic technique. Deposit the disks so that their centres are no less than 24 mm apart. The maximal number of discs applied on 90mm plate is 6 discs, on 150mm plate- 12 discs. In order to estimate the induction resistance of Streptococci and Staphylococci to Clindamycin, the Erythromycin and Clindamycin discs should be applied 12-20 mm from the edge of Petri dish.
4. Within 15 minutes place the dishes in incubator and incubate in conditions as per EUCAST recommendations (table below).

Media, temperature, conditions and time of incubation recommended by EUCAST

Testing microorganisms	Medium	Incubation temperature	Incubation conditions	Incubation time
Enterobacteriaceae	MH Agar (MH)	35 °C ± 1	Aerobic	16 – 20 h
Pseudomonas spp.	MH Agar (MH)	35 °C ± 1	Aerobic	16 – 20 h
Stenotrophomonas maltophilia	MH Agar (MH)	35 °C ± 1	Aerobic	16 – 20 h
Acinetobacter spp.	MH Agar (MH)	35 °C ± 1	Aerobic	16 – 20 h
Staphylococcus spp.	MH Agar (MH)	35 °C ± 1	Aerobic	16 – 20 h
Enterococcus spp.	MH Agar (MH)	35 °C ± 1	Aerobic	16-20 h; 24 h for glycopeptides
Streptococcus spp. Group A,B,C,G	MH+5% Horse Blood +20 mg/l NAD (MH-F)	35 °C ± 1	5 % CO_2	16 – 20 h
Streptococcus pneumoniae	MH+5% Horse Blood+ 20 mg/l NAD (MH-F)	35 °C ± 1	5 % CO_2	16 – 20 h
Streptococcus spp. Group *viridans*	MH+5% Horse Blood +20 mg/l NAD (MH-F)	35 °C ± 1	5 % CO_2	16 – 20 h
Haemophilus spp.	MH+5% Horse Blood +20 mg/l NAD (MH-F)	35 °C ± 1	5 % CO_2	16 – 20 h
Moraxella catarrhalis	MH+5% Horse Blood +20 mg/l NAD (MH-F)	35 °C ± 1	5 % CO_2	16 – 20 h
Listeria monocytogenes	MH+5% Horse Blood +20 mg/l NAD (MH-F)	35 °C ± 1	5 % CO_2	16 – 20 h
Pasteurella multocida	MH+5% Horse Blood +20 mg/l NAD (MH-F)	35 °C ± 1	5 % CO_2	16 – 20 h
Campylobacter jejuni and *coli*	MH+5% Horse Blood +20 mg/l NAD (MH-F)	41 °C ± 1	microaerophilic conditions	24 h, max. up to 40-48h
Corynebacterium spp. except *Corynebacterium diphtheria*	MH+5% Horse Blood +20 mg/l NAD (MH-F)	35 °C ± 1	5% CO_2	16 – 20 h, max. up to 40-48h

INTERPRETATION
Interpret the measured zones of inhibition in reference to published tables (EUCAST, CLSI, literature). Zones are measured to the nearest whole millimetre. Measurements may be simplified by software BioStrefa LITE available in BioMaxima.

NOTES
1. For professional use, in vitro diagnostic only.
2. The disks should be used according to an appropriate standardised method. Any deviation from the prescribed method may result in false results.
3. Accuracy of the tests depends on the disk potency, proper inoculum, inoculation technique, incubation temperature, medium plates (nature of medium and its depth) etc.
4. Quality control of the susceptibility tests should be routinely performed with the reference cultures specified. If the disks form incorrect zones with recommended control bacteria you should check the entire procedure.
5. It is recommended to use updated interpretative standards as a response to the dynamics of resistance.
6. The reference strains from ATCC collection certified with FDA and CE are available in BioMaxima S.A.

REFERENCES

1. Bauer AW, Kirby WMM, Sherris JC, Tuck M: Am J Clin Pathol 1966; 45: 492-496.
2. Federal Register. Rules and regulations. Antibiotic susceptibility dicss. Fed Regist 1972; 37: 20525-20529.
3. World Health Organization Expert Committee on Biological Standardization. Requirements for antibiotic susceptibility test: 1.: WHO Technical reports series No 610. Geneva: WHO, 1977.
4. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters. Version 4.0, January, 2014.

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CE

Explanation of symbols

⚠	Caution, consult accompanying documents		Consult instructions for use		Manufacturer
IVD	For in vitro diagnostic use	LOT	Batch code	REF	Catalog number
	Temperature limitation		Use by		

BioMaxima

BioMaxima S.A.
ul. Vetterów 5
20-277 Lublin
Poland

phone:+48 81 745 51 40
fax: +48 81 744 29 15
info@biomaxima.com
www.biomaxima.com