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Novobiocin NV 5 mcg

Novobiocin NV 5 mcg are used for antimicrobial susceptibility testing of bacterial cultures as per Kirby-Bauer Method.

REF: BS.1/NV01.050.0050	50 Discs	REF: BS.1NV05.050.0250	250 Discs

METHOD PRINCIPLE

Antimicrobial Susceptibility Testing (AST) is a laboratory procedure performed by laboratory technician to identify, which antimicrobial regimen is specifically effective for individual patients. The introduction of various antimicrobials for treating variety of infections showed the necessity of performing antimicrobial susceptibility testing as a routine procedure in all microbiology laboratories. antibiotics are generally defined as the substances produced by the microorganism such as Penicillium, which has the ability to kill or inhibit the growth of other microorganisms, mainly bacteria. Antimicrobial Susceptibility Tests (ASTs) basically measures the ability of an antibiotic or other antimicrobial agent to inhibit the In vitro microbial growth. The basic principle of the antibiotic susceptibility testing has been used in microbiology laboratories over 80 years. Till the 1950s, laboratories were lacking in the methodologies and equipment's for the accurate determination of In vitro responses of organisms to antimicrobial agents. Bauer et al., began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. Antimicrobial Susceptibility Tests are either quantitative or qualitative. Clinical laboratories currently employ several methods depending on the laboratory test menu that they provide. These approaches include the disk diffusion and Minimum Inhibitory Concentration (MIC) methods. 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. Various regulatory agencies and standards-writing organizations, published standardized reference procedures 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) for any antimicrobial testing, Quality control or clinical testing. However, few precautions are to be maintained while

COMPOSITION

Item	Concentration
Novobiocin	5 mcg

PRECAUTIONS AND WARNING

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 environment contamination.

S61: avoid release in environment.

For further information, refer to the Novobiocin 5 mcg material safety data sheet.

STORAGE AND STABILITY

BioScien Novobiocin 5 mcg 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

Prepare plates with Mueller Hinton Agar (201130650100 / 201130650500, 203130830250 / 203130830100, 203090070015 / 203090070030 & Ready Prepared Plates – 205131790100 / 205131790020) for use in the Kirby-Bauer Method for rapidly growing aerobic organisms. For fastidious organisms such as Streptococci, the agar (201130650100 / 201130650500) is supplemented with 5% sterile, defibrinated blood. For Haemophilus spp., Haemophilus Test Medium (for all agents when testing H. influenzae or H. parainfluenzae) or MH-F agar (MHA with 5% mechanically defibrinated horse blood and 20 µg/mL NAD) (for selected agents when testing H. influenzae); for N. gonorrhoeae, GC Agar Base (201070010500) with 1% defined growth supplement and for Streptococcus pneumoniae, MHA with 5% sheep blood or MH-F agar (MHA with mechanically defibrinated horse blood and 20 µg/mL NAD) are recommended respectively. The medium in the plates should be sterile and have a depth of about 4 mm.

Bacterial Inoculum Preparation

1. Gram staining is done before starting susceptibility testing to confirm the culture purity and to determine appropriate battery of tests.

2. Select 3-4 colonies from subcultured slant or plate and transfer them into 5 mL of sterile 0.85% saline, vortex the suspension and adjust the turbidity to yield a uniform suspension matching 0.5 McFarland standard (O.D at 630 nm range: 0.08-0.130). Note: The direct colony suspension method is preferred for Staphylococcus spp., S. pneumoniae and other Streptococci, Haemophilus spp. and N. gonorrhoeae. OR 3-5 well-isolated colonies should be selected and transferred to 4-5 mL of a suitable broth using an inoculating needle.

3. Incubate the broth (usually 2-6 hours) at 35°C-37°C until it achieves or exceeds the turbidity of the 0.5 McFarland's barium sulphate standard. This results in a suspension containing approximately 1-2 X 108 cfu/mL. Note: Overnight broth cultures should not be used as inoculum.

4. Adjust the turbidity to the barium sulphate standard. For the diluents use sterile broth or sterile saline. The turbidity of the standard and the test inoculums should be compared by holding both tubes in front of a white background with finely divided lines or by use of a photometric device.

5. Within 15 minutes of adjusting the turbidity of the inoculum, immerse a sterile cotton swab into the properly diluted inoculum and rotate it firmly several times against the upper inside wall of the tube to express excess fluid.

6. Inoculate the entire agar surface of the plate three times, rotating the plate 60° between streaking to obtain even inoculation. Swab the rim of the agar bed too.

7. The lid may be left ajar for 3-5 minutes and the plate held at room temperature for not more than 15 minutes to allow the surface moisture to be absorbed before applying the antibiotic discs.

8. Apply discs by means of an antimicrobial disc dispenser, aseptically, at least 24 mm apart. Preferably, deposit Penicillin and Cephalosporin discs not more than 10 mm from the edge of the Petri dish, and their centers at least 30 mm apart. Avoid placing such discs adjacent to one another. Tap the discs with some sterile needle or forceps after placing them on the agar for complete contact with the medium surface.

9. Within 15 minutes of applying the discs, invert the plates and incubate at 35°C-37°C. With non-fastidious organisms the plates should not be incubated under an increased concentration of carbon dioxide. Note: For Staphylococcus spp., testing at temperatures above 35°C may not detect Methicillin-resistant Staphylococci (MRS); for N. gonorrhoeae, incubate at 35°C-37°C [do not exceed 37°C]. Haemophilus spp., N. gonorrhoeae, S. pneumoniae and other Streptococci should be incubated in an atmosphere enriched with 5% CO_2.

10. Examine plates after 18-24 hours of incubation. 20-24 hours for N. gonorrhoeae, S. pneumoniae and other Streptococci). A full 24 hours of incubation is recommended to detect methicillin/ oxacillin/ vancomycin-resistant Staphylococci in Staphylococcus spp., cefoxitin in Staphylococcus spp. (except from S. aureus, S. lugdunensis, S. pseudintermedius and S. schleiferi), and Enterococcus spp. in vancomycin resistance. The diameters of the zones of complete inhibition are measured, as determined by gross visual inspection.

Zones are measured to the nearest whole millimeter. For further details in measuring zones of inhibition, consult the reference. If only isolated colonies grow, the inoculum is too light and the test should be repeated. Zones around discs containing different drugs are not comparable for the purpose of comparing activity of drugs. See the Zone Diameter Interpretive Chart, which gives expected values from testing common aerobes.

EQUIPMENT 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)*
Staphylococcus aureus subsp. aureus (25923)	22-31

REFERENCES

1.Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, CLSI Vol. 34, Feb 2024.
3. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use		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		