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HARDY DIAGNOSTICS
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Instructions for Use

HIGH-LEVEL AMINOGLYCOSIDE RESISTANCE (HLAR) DIFFERENTIATION DISKS

Gentamicin, 120mcg	50 disks/cartridge
Streptomycin, 300mcg	50 disks/cartridge

INTENDED USE

Gentamicin and Streptomycin High-Level Aminoglycoside Resistance (HLAR) Differentiation Disks, are used to detect high-level aminoglycoside resistance in *Enterococcus faecalis* and *E. faecium*. These disks are designated for use for HLAR testing, in accordance with the NCCLS performance standards for susceptibility testing.[8,9]

SUMMARY

Enterococci are characteristically resistant to a wide variety of antimicrobial agents making single-drug therapy often ineffective. Systemic enterococcal infections, such as endocarditis, are usually treated with a combination of two antimicrobial agents: one specific action against the cell wall, such as a beta-lactam or a glycopeptide (i.e., penicillin, ampicillin or vancomycin) and an aminoglycoside, which inhibits bacterial protein synthesis (i.e., gentamicin or streptomycin). These agents act synergistically to enhance killing of the bacteria, since the aminoglycoside has increased uptake into the cell, after cell wall damage by the beta-lactam agent.[2,3,5]

All enterococci naturally have low-level resistance to aminoglycosides, which invalidates use of the disk test with usual concentrations of antimicrobial agents. HLAR is only meaningful for a testing method. When an enterococcal strain has high-level resistance to the aminoglycoside, there is no synergism and combination therapy with a beta-lactam drug will not have the desired bactericidal effect. Therefore, it is important to detect the presence of high-level resistance in order to predict aminoglycoside synergy.[2]

Strains that show HLAR to gentamicin, the most commonly used and best aminoglycosides against enterococci, possess one or more aminoglycoside-modifying enzymes. These enzymes may make them resistant to one or more of a variety of other aminoglycosides, including tobramycin, netilmicin, and amikacin, but not streptomycin.[5] Other HLAR enzymes are active against streptomycin, but not gentamicin. Thus, testing gentamicin and streptomycin is preferred to provide information on the two most active of the aminoglycosides, that do not show cross-resistance to each other. If a strain has high-level resistance to both gentamicin and streptomycin, tobramycin will not be effective. There are some strains that have emerged that will still be amikacin or netilmicin susceptible, however, and testing for high-level resistance in these agents may be indicated.[12]

Many isolates of *E. faecalis* and *E. faecium* have acquired high-level resistance to one or more of the aminoglycosides, while other enterococci and viridans streptococci have not yet acquired the genes for resistance. Performing *in vitro* susceptibility testing of clinical isolates of *E. faecalis* and *E. faecium*from systemic infections is critical for determining which combination of agents may be effective therapy.[3]

The three most commonly used methods for HLAR detection are; agar dilution, broth microdilution, and disk diffusion

(using high-concentration disks). When performing the disk diffusion test to determine high-level resistance to aminoglycosides, it is important to remember that standard gentamicin and streptomycin disks (10mcg each) that are used for routine disk diffusion testing cannot be used. Only high-concentration disks can be used to determine aminoglycoside resistance.[5]

FORMULA

Each HLAR Differentiation Disks contains the following specified concentrations of the appropriate antibiotics on high quality 6mm diameter filter paper disks:

Gentamicin	120mcg
Streptomycin	300mcg

STORAGE AND SHELF LIFE

Storage: Upon receipt store at -20ºC. away from direct light. A small supply of disks for use within one week can be stored at 4ºC. The disks should not be used if there are any signs of deterioration, discoloration, or if the expiration date has passed. Protect from light, excessive heat, and moisture.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: This product is not intended for the primary isolation of patient specimens. It should be used only with cultures of isolated organism. **These are high-concentration disks; do not use standard susceptibility disks for HLAR testing.**

1. Allow disks to equilibrate to room temperature.

2. Using a pure 18-24 hour culture, prepare a suspension (equivalent to a McFarland 0.5 opacity standard) of the organism to be tested.

3. Dip a sterile non-toxic cotton swab (Cat. no. 258061WC) into the organism suspension. Rotate the swab several times, pressing firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab. Evenly inoculate the dried surface of a Mueller Hinton Agar plate (Cat. no. H11 or G45) by streaking the swab over the entire surface of the plate in three directions, as for a routine disk diffusion test.[9]

4. Aseptically place one Gentamicin Disk (120mcg) and one Streptomycin Disk (300mcg) on the media surface, far enough away from each other to leave room for zones of inhibition (greater than 30mm apart). With sterile forceps, gently tap each disk to the media surface to ensure uniform diffusion of the antibiotic into the medium.

5. Invert plates, and incubate aerobically at 35ºC. for 18-24 hours.

6. Examine plates for confluent or almost confluent growth, and measure zones of inhibition for each disk. (If growth is unacceptable, the test cannot be interpreted). Reincubation of the plate for an additional 24 hours may be done to verify susceptibility of the strain to streptomycin.

INTERPRETATION OF RESULTS

Sensitive: Zone size is greater than or equal to 10mm. The organism is not an HLAR *Enterococcus* and synergy with the cell wall-active agent is likely.

Resistant: No zone of inhibition (6mm). The organism is an HLAR *Enterococcus* and synergy with the cell wall-active agent is not likely.

Intermediate: Zone sizes of 7 to 9mm are considered inconclusive, and should be retested by an alternative method (i.e., standard agar screen or broth microdilution methods).[2,4,5]

LIMITATIONS

Zone sizes of 7 to 9mm should be tested by an alternative method, as problems exist with detection of this resistance by commercial susceptibility systems.[6]

Standard gentamicin (10mcg) and streptomycin (10mcg) disks that are used for routine disk diffusion testing are not intended to be used for determining high-level aminoglycoside resistance (HLAR).[5]

Some isolates with HLAR to streptomycin may not demonstrate resistance until after 48 hours of incubation.[5]

All *Enterococcus faecium* strains produce a chromosomally encoded aminoglycoside acetyltransferase, which eliminates synergism between cell wall-active antimicrobials and the aminoglycosides tobramycin, kanamycin, netilmicin, and sisomicin. Performing further testing for resistance to these agents is not meaningful.[12]

Susceptibility to either gentamicin or streptomycin does not predict susceptibility to other aminoglycosides; erroneous therapeutic decisions can result if cross-susceptibility is assumed.

Strains that are high-level resistant to gentamicin are also resistant to tobramycin and kanamycin, but may still be susceptible to amikacin (or netilmicin, for *E. faecalis*). If the strain is resistant to both gentamicin and streptomycin, further testing for high-level resistance to amikacin and netilmicin in a reference laboratory may be indicated.[12]

Resistance to streptomycin does not predict resistance to any other aminoglycoside.

Mueller Hinton Agar supplemented with sheep blood is not appropriate for determining HLAR, since larger zones of inhibition were observed than those observed on unsupplemented agar, possibly causing false susceptibility, especially with streptomycin.[11]

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Zone Size	
		Time	Temperature	Atmosphere	Gentamicin	Streptomycin
Enterococcus faecalis ATCC® 51299	F	18-24hr	35°C	Aerobic	6mm	6mm
Enterococcus faecalis ATCC® 29212	F	18-24hr	35°C	Aerobic	16-23mm	14-20mm

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

HLAR Differentiation Disks are 6mm (in diameter) filter paper disks, and should appear white in color.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

4. Isenberg, H.D. 1998. Chap. 5 - Antimicrobial Susceptibility Testing, p. 227-229. *Essential Procedures for Clinical Microbiology*. American Society for Microbiology, Washington, D.C.

5. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

6. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

7. Bantar, C.E., M. Micucci, L. Fernandez Canigia, J. Smayevsky, and H.M. Bianchini. 1993. Synergy Characterization for *Enterococcus faecalis* Strains Displaying Moderately High-Level Gentamicin and Streptomycin Resistance. *J. Clin. Microbiol.*; 31:1921-1923.

8. NCCLS, January 2003. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*; approved standard 6th ed. M7-A6.

9. NCCLS, January 2003. *Performance Standards for Antimicrobial Disk Susceptibility Tests*; approved standard 8th ed. M2-A8.

10. Sahm, D.F., and C. Torres. 1988. High-Content Aminoglycoside Disks for Determining Aminoglycoside-Penicillin Synergy against *Enterococcus faecalis*. *J. Clin. Microbiol.*; 26:257-260.

11. Swenson, J.M., M.J. Ferraro, D.F. Sahm, N.C. Clark, D.H. Culver, F.C. Tenover, and The National Committee for Clinical Laboratory Standards study group on Enterococci. 1995. Multilaboratory Evaluation of Screening Methods for Detection of High-Level Aminoglycoside Resistance in Enterococci.*J. Clin. Microbiol.* ; 33:3008-3018.

12. Chow, J.W. 2000. Aminoglycoside Resistance in Enterococci. *Clin. Infect. Dis*. 31:586-589.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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