remel

MUELLER HINTON AGAR w/ and w/o 5% SHEEP BLOOD

INTENDED USE

Remel Mueller Hinton Agar w/ and w/o 5% Sheep Blood are solid media recommended for use in qualitative procedures in the standardized antimicrobial disk diffusion susceptibility test by the Kirby-Bauer method.

SUMMARY AND EXPLANATION

Mueller Hinton Agar was developed in 1941 for the cultivation of pathogenic *Neisseria* spp. In 1966, Bauer et al. adopted the use of Mueller Hinton for antimicrobial susceptibility testing.[1,2] They emphasized the importance of using standardized methods to increase the predictive value of single disk concentration procedures. Their recommendations included the use of Mueller Hinton Agar at a specified depth, an inoculum adjusted to the turbidity of a 0.5 McFarland, and proper inoculation and incubation techniques. Mueller Hinton Agar is recommended by the Clinical and Laboratory Standards Institute (CLSI) for routine susceptibility testing of nonfastidious bacteria.[3]

PRINCIPLE

Mueller Hinton Agar contains beef extract and acid hydrolysate of casein which supply amino acids, nitrogenous substances, vitamins, and minerals necessary for growth. The starch present acts as a protective colloid against toxic materials present in the medium. The medium contains low levels of thymidine and thymine, as excess amounts can reverse the inhibitory effect of sulfonamides and trimethoprim. The calcium and magnesium levels are controlled so appropriate activity of aminoglycosides, tetracycline, and colistin can be expected when testing *Pseudomonas aeruginosa*. Agar is the solidifying agent. Sheep blood is added to enhance the growth of *Streptococcus pneumoniae.*

In the disk diffusion test, disks impregnated with a specific concentration of antibiotic are placed on the surface of an inoculated Mueller Hinton Agar plate. The antibiotic diffuses into the agar creating a gradually changing gradient of the drug concentration around the disk. If the organism is susceptible, the microbial growth around the periphery of the disk is inhibited. If the organism is resistant, growth is not inhibited. The zones of inhibition are measured, correlated with minimum inhibitory concentration (MIC) values, and compared to CLSI interpretative criteria to determine the degree of susceptibility.

REAGENTS (CLASSICAL FORMULA)*

Acid Digest of Casein	17.5	g
Beef Extract	2.0	g
Starch	1.5	g
Sheep Blood (optional)	5	%
Agar	17.0	g
Demineralized Water	1000.0	ml

pH 7.3 ±- 0.1 @ 25°C

*Adjusted as required to meet performance standards.

PRECAUTIONS

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Do not incubate prior to use.

PRODUCT DETERIORATION

This product should not be used if (1) there is evidence of dehydration, (2) the product is contaminated, (3) the color has changed, (4) the expiration date has passed, or (5) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.[4,5]

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) 0.5 McFarland standard (R20410) or equivalent photometric device (optional), (7) Antimicrobial susceptibility disks, dispensing apparatus, (8) Sliding caliper, ruler, or template, (9) CLSI *Performance Standards for Antimicrobial Susceptibility Testing; Informational Supplement* (current edition).

PROCEDURE

Note: Use Mueller Hinton Agar for routine testing of nonfastidious organisms. Mueller Hinton Agar w/ 5% Sheep Blood should be used to test strains that fail to grow satisfactorily on plain Mueller Hinton, such as *Streptococus* spp. Media requirements for fastidious or infrequently isolated bacteria can be found in CLSI document M45.[6]

1. Implement appropriate test methods to confirm presumptive identification of the test isolate.
2. Allow plates and antimicrobial susceptibility disks to equilibrate to room temperature before use. The agar surface should not have excess moisture prior to inoculation.
3. Inoculum may be prepared utilizing either the growth method or the direct colony method. Refer to appropriate CLSI documents for guidelines specific to each test organism.[3,6]
   a. Growth Method:
      i. Select at least 3-5 isolated colonies of the same morphological type from an agar culture. Touch the top of each colony with a loop and transfer to 4-5 ml of a suitable broth medium such as Tryptic Soy Broth (TSB).
      ii. Incubate the broth at 35-37°C until the turbidity equals or exceeds that of a 0.5 McFarland standard or equivalent.
      iii. If necessary, adjust the turbidity of the suspension with sterile saline or broth to achieve a turbidity equivalent to a 0.5 McFarland standard. This step may be accurately performed visually or using a photometric device. If performed visually, use adequate light to compare the suspension to the 0.5 McFarland standard against a card with a white background and contrasting black lines.[3]
   b. Direct Colony Method:
      (Method of choice for *Streptococcus* and *Staphylococcus* spp.)
      i. Prepare a direct suspension of the test organism in saline or broth from an 18-24 hour culture on nonselective media.
      ii. Adjust the turbidity of the suspension as described under Growth Method.
4. Inoculate agar plates within 15 minutes of preparing organism suspension.
5. Immerse a sterile swab into the suspension and rotate against the side of the tube above the fluid level, to remove excess fluid.
6. Inoculate the surface of the plate in three planes by rotating the plate approximately 60 degrees each time.
7. Replace the lid and allow the plate to rest on the bench at least 3 minutes but no longer than 15 minutes for the inoculum to be absorbed before applying antimicrobial susceptibility disks.
8. Apply disks individually or by using an antimicrobial disk dispenser. Disks should be no closer than 24 mm from center to center and not placed too close to edge of plate. Because some drug diffuses almost instantaneously, do not relocate a disk once it has come in contact with the agar surface. Tap disks gently with a sterile needle or forceps to ensure complete contact with the agar surface. (Mueller Hinton Agar: do not apply more than 12 disks per 150 mm plate or 5 disks per 100 mm plate; Mueller Hinton Agar w/ 5% Sheep Blood: do not apply more than 9 disks per 150 mm plate or 4 disks per 100 mm plate.)
9. Invert the plate and place in the incubator within 15 minutes of disk application.
   a. Incubate Mueller Hinton Agar plates in ambient air at 35-37°C for 16-18 hours.
      Note: Staphylococci and enterococci require a full 24 hours of incubation. Methicillin-resistant staphylococci (MRS) may not be detected at incubation temperatures above 35°C.
   b. Incubate streptococci in 5-7% CO_2 at 35-37°c for 20-24 hours.
   c. Refer to CLSI document M45 for incubation times, temperatures, and atmospheres for fastidious or infrequently isolated bacteria.[6]

Mueller Hinton Agar Deep: Melt the agar deep in a boiling water bath and cool to 45-50°C. Mix and dispense into a sterile Petri dish and proceed with the instructions above.

INTERPRETATION

1. After incubation, examine plate(s) for a confluent lawn of growth and uniformly circular zones of inhibition around the antimicrobial disks. If individual colonies are present, the inoculum was too light and the procedure must be repeated.
2. Using a sliding caliper or ruler, measure the diameters of the zones of complete inhibition (including the disk) to the nearest whole millimeter. Zone margins should be read as the area showing no obvious growth that is detectable with the unaided eye. Faint growth or tiny colonies at the edge of the zone of inhibited growth, only detectable by magnification, should be disregarded.
   Mueller Hinton Agar: Invert the plate and position a few inches above a black, non-reflecting background illuminated with reflected light. Place the measuring device on the back of the inverted plate. If the test organism is a *Staphylococcus* or an *Enterococcus* spp., transmitted light (plate held up to light) is used to examine for light growth of methicillin- or vancomycin-resistant strains within apparent zones of inhibition. Any discernible growth within the zone of inhibition for these organisms is indicative of methicillin- or vancomycin-resistance. Swarming within the zone of inhibition may occur for some *Proteus* spp., but zones are usually well-defined, and the thin veil of swarming should be ignored.
   Mueller Hinton Agar w/ 5% Sheep Blood: Remove the cover and measure the zones of inhibition from the upper surface of the agar, illuminated with reflected light.
3. Interpret zones of inhibition by referring to the most current CLSI Tables (M100) for each organism.[7] Report the isolate susceptibility profile accordingly, paying special attention to all applicable footnotes and recommendations for further testing of resistant isolaes.[3, 6-8]

QUALITY CONTROL

All lot numbers of Mueller Hinton Agar w/ and w/o 5% Sheep Blood have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing conforms with or exceeds CLSI standards.[3,9] Controls should be included each time a susceptibility test is performed or weekly if satisfactory performance can be documented according to CLSI standards. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Mueller Hinton Agar:		
**Enterococcus faecalis* ATCC® 33186	Ambient, 24 h @ 35°C	Zone sizes within current CLSI guidelines
**Escherichia coli* ATCC® 25922	Ambient, 16-18 h @ 35°C	Zone sizes within current CLSI guidelines
**Escherichia coli* ATCC® 35218	Ambient, 16-18 h @ 35°C	Zone sizes within current CLSI guidelines
**Pseudomonas. aeruginosa* ATCC® 27853	Ambient, 16-18 h @ 35°C	Zone sizes within current CLSI guidelines
**Staphylococcus aureus* ATCC® 25923	Ambient, 24 h @ 35°C	Zone sizes within current CLSI guidelines
Mueller Hinton Agar w/ 5% Sheep Blood:		
**Streptococcus pneumoniae* ATCC® 49619	CO_2, 20-24 h @ 35°C	Zone sizes within current CLSI guidelines

*CLSI recommended organism

LIMITATIONS

1. This media is not for use with anaerobes, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, or other pathogenic microorganisms not listed in the current CLSI documents.
2. Clinical microbiology laboratories should regularly monitor procedures for technical human errors that may compromise the accuracy of disk diffusion results. Such errors include but are not limited to: improper disk storage, inoculum not properly adjusted, improper incubation temperatures, times, and/or atmospheres, transcription and reading errors when measuring zone diameters, and contamination or mutation in the control strains.[3,6]
3. Zones of inhibition may vary when using Mueller Hinton Agar w/ 5% Sheep Blood. Oxacillin and methicillin zones may be slightly smaller (2-3 mm). Sheep blood can cause indistinct zones or a film of growth within the zones around sulfonamide and trimethoprim disks; measure the more obvious margin of growth. Do not use nafcillin on blood containing media.[3]
4. Some organism-antimicrobial agent combinations should not be reported since susceptible results may be misleading. Refer to CLSI documents and standard references for reporting guidelines specific to each organism-antimicrobial agent combination.[5,7]
5. Refer to CLSI documents for a complete discussion on the testing and detection of methicillin and vancomycin resistance in staphylococci, resistant enterococci and *S. pneumoniae*, extended-spectrum β-lactamase-producing (ESBL) gram-negative bacilli, and other test limitations.[3, 6-8]
6. Disk diffusion methods have been standardized for rapidly growing pathogens, such as *Staphylococcus* spp., the *Enterobacteriaceae*, *Enterococcus* spp, and *S. pneumoniae*, among others. They have been modified to accommodate fastidious or infrequently isolated bacteria. Refer to CLSI documents M2 and M45 for testing guidelines specific to each isolate. [3,6]

BIBLIOGRAPHY

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5. Isenberg, H.D. 2004. Clinical Microbiology Procedures Handbook. 2nd ed., Vol. 2. ASM Press, Washington, D.C.
6. Clinical and Laboratory Standards Institute. 2006. Methods for Anti-microbial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline. M45-A. CLSI, Wayne, PA.
7. Clinical and Laboratory Standards Institute. 2012. Performance Standards for Antimicrobial Susceptibility Testing; 22nd Informational Supplement. M100-S22. CLSI, Wayne, PA.
8. Clinical and Laboratory Standards Institute. 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard, 9th ed. M7-A9. CLSI, Wayne, PA.
9. Clinical and Laboratory Standards Institute. 2006. Protocols for Evaluating Dehydrated Mueller-Hinton Agar; Approved Standard, 2nd ed. M6-A2. CLSI, Wayne, PA.

PACKAGING

Mueller Hinton Agar:

REF R01620, 15 X 100 mm Plate..... 10/Pk
REF R01624, 15 X 100 mm Plate..... 100/Cs
REF R04050, 15 X 150 mm Plate..... 10/Pk
REF R04052, 15 X 150 mm Plate..... 40/Cs
REF R09592, Deep, 25 ml/Tube 20/Pk

Mueller Hinton Agar w/ 5% Sheep Blood:

REF R01622, 15 X 100 mm Plate..... 10/Pk
REF R01623, 15 X 100 mm Plate 100/Cs
REF R04055, 15 X 150 mm Plate..... 10/Pk
REF R04057, 15 X 150 mm Plate..... 40/Cs

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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