



MUELLER HINTON AGAR WITH 5% SHEEP BLOOD

- For in vitro use only -

Catalogue No. PM91 (K)

Our Mueller Hinton Agar with 5% Sheep Blood is the recommended medium for the antimicrobial disk diffusion testing of common, aerobic, rapidly growing bacteria.

Mueller Hinton was originally devised as a medium for culturing *Neisseria* species. Later on in the 1960's, Kirby, Bauer and other researchers attempted to standardize the procedure used in susceptibility testing of bacteria and selected Mueller Hinton Agar as the ideal medium. The Kirby-Bauer procedure is based on the use of paper disks impregnated with antimicrobial agents. The antimicrobial agents diffuse into the agar medium resulting in various zones of inhibition depending on the organism being tested. Today, the Clinical and Laboratory Standards Institute (CLSI) puts forth the standardized guidelines for the use and interpretation of Mueller Hinton Agar. The CLSI document M2, *Performance Standards for Antimicrobial Disk Susceptibility Tests*, provides procedures for the testing of rapidly growing aerobic and facultatively anaerobic bacteria, which include members of the *Enterobacteriaceae*, *Staphylococcus* spp., *Enterococcus* spp., *Pseudomonas* spp., *Acinetobacter* spp., and *Vibrio cholerae*. Modified mediums and testing procedures are also provided for more fastidious species such as *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and *Streptococcus pneumoniae*.

The CLSI medium of choice for susceptibility testing of *Streptococcus pneumoniae* is Mueller Hinton Agar supplemented with 5% defibrinated sheep blood. *Streptococcus pneumoniae* historically was susceptible to penicillins and many other antimicrobial agents. A developing body of both laboratory and clinical evidence indicates that this is no longer true. Data from the literature indicates that in some countries as many as 40% of strains are intermediate or resistant to penicillin. Along with penicillin, resistance has emerged to other agents as well, including cephalosporins, macrolides and co-trimoxazole. It is now essential

that laboratories test strains of *S. pneumoniae* for resistance to these agents in defined circumstances.

Formulation per Litre of Medium

| | |
|--------------------------------|---------|
| Beef Extract | 2.0 g |
| Casein Hydrolysate | 17.5 g |
| Starch | 1.5 g |
| Agar | 17.0 g |
| Defibrinated Sheep Blood | 50.0 mL |

pH 7.3 ± 0.1

Recommended Procedure

1. Mueller Hinton Agar with 5% Sheep Blood plates and the required antibiotic disks should be removed from the refrigerator or freezer 1 to 2 hours before use so that they may equilibrate to room temperature. Remove plates from packaging to allow excess moisture to dissipate from the surface during this warm-up period.
2. Growth from a pure, overnight culture, grown on sheep blood agar, is suspended in Mueller-Hinton or 0.9% saline to a density equivalent to the turbidity of a 0.5 McFarland Standard (equivalent to 1.5×10^8 CFU/mL). This suspension must be used within 15 minutes of its adjustment to ensure accuracy.
3. Dip a sterile swab into the suspension and roll the swab firmly against the side of the tube several times to remove excess inoculum from the swab.
4. Use the swab to streak the entire surface of the Mueller Hinton Agar plate. Repeat the streaking procedure two more times, rotating the plate approximately 60° each time to ensure even distribution of the inoculum. The inoculated plate may be left for 5 minutes to allow the surface inoculum to be adsorbed.

5. Using aseptic technique, the selected antimicrobial disks are evenly distributed on the agar surface individually or with a dispensing apparatus. Press down gently on each disk to ensure that complete contact with the agar surface is obtained.
6. Invert plates and incubate at 35°C in a 5 to 7% CO₂-enriched environment for 20 to 24 hours.
7. After the incubation period, examine and interpret plates.

Interpretation of Results

After the incubation period, a confluent lawn of growth should be obtained; if individual colonies are observed instead than the inoculum was too light and the test must be repeated. A zone of inhibition should be observed around each antimicrobial disk. Using a ruler or calipers measure the zones from the upper surface of the agar illuminated with reflected light and the cover removed. The zone margin or endpoint should be taken as the area showing no obvious visible growth that can be detected with the unaided eye. Disregard faint growth of tiny colonies that can be detected with difficulty near the edge of the obvious zone of inhibition. However, discrete colonies growing within a clear zone of inhibition should be subcultured, re-identified and retested.

To accurately determine susceptibility results use updated CLSI tables of antimicrobial disks and interpretative standards. The complete standard and informational supplements can be ordered directly from the Clinical and Laboratory Standards Institute; visit clsi.org for more information.

- *Numerous factors can affect results and zone sizes: inoculum size; rate of growth; pH; length of incubation and incubation environment; disk content and drug diffusion rate; and measurement of endpoints. Therefore strict adherence to CLSI testing protocols are essential for obtaining accurate and reproducible results.*
- *A maximum of 4 antimicrobial disks are prescribed for the regular 100-mm size plates, while no more than 9 disks should be placed on the Kirby 150-mm plates.*

Quality Control

Internal monitoring and testing of each lot of Mueller Hinton Agar is required as outlined by the CLSI. Each time a new lot of agar or a new lot of antimicrobial disks is introduced, it must be tested with the appropriate quality control strains. For more information please refer to The CLSI document M2, *Performance Standards for Antimicrobial Disk Susceptibility Tests*.

Storage and Shelf Life

Our Mueller Hinton Agar with 5% Sheep Blood should be stored at 4°C to 8°C and protected from light. The medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions the medium has a shelf life of 8 weeks from the date of manufacture.

Ordering Information

| Cat# | Description | Format |
|-------|--|--------|
| PM91 | Mueller Hinton Agar with 5% Sheep Blood [Standard 15x100-mm plate] | 10/pkg |
| PM91K | Mueller Hinton Agar with 5% Sheep Blood [Kirby 15x150-mm plate] | 5/pkg |

References

1. Mueller JH, Hinton J. A protein-free medium for primary isolation of Gonococcus and Meningococcus. Proc Soc Exp Biol Med 1941; 48:330-3.
2. Bauer AW, Sherris JC. The determination of sulfonamide susceptibility of bacteria. Chemotherapy 1964; 9:1-19.
3. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized disc method. Am J Clin Pathol 1966; 49:3-6.
4. Reller LB, Schoenknecht FD, Kenny MA, Sherris JC. Antibiotic susceptibility testing of Pseudomonas aeruginosa: selection of a control strain and criteria for magnesium and

- calcium content in media. J Infect Dis 1974; 130:454-63.
5. Ferone R, Bushby SR, Burchall JJ, Moore WD, Smith D. Identification of Harper-Cawston factor as thymidine phosphorylase and removal from media substances interfering with susceptibility testing to sulfonamides and diaminopyrimidines. Antimicrob Agents Chemother 1975; 7:91-8.
 6. Murray PR, Tenover FC, Tenover FC. Evaluation of Mueller-Hinton agar for disk diffusion susceptibility tests. J Clin Microbiol 1983; 18
 7. MacFaddin JF. Media for isolation, cultivation, identification, maintenance of bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
 8. Jenkins RD, Stevens SL, Craythorn JM, Thomas TW, Guinan ME, Matsen JM. False susceptibility of enterococci to aminoglycosides with blood-enriched Mueller-Hinton agar for disk susceptibility testing. J Clin Microbiol 1985; 22:369-74.
 9. NCCLS. Protocols for evaluating dehydrated Mueller-Hinton agar; approved standard. M6-A. Wayne, PA: NCCLS, 1996.
 10. Centers for Disease Control and Prevention. Morbidity and Mortality Weekly Report. Defining the public health impact of drug-resistant *S. pneumoniae*: Report of a working group. MMWR 1996; 45: 1-20.
 11. Bradley JS, Scheld WM. The challenge of penicillin resistant *Streptococcus pneumoniae* meningitis: current antibiotic therapy in the 1990s. Clin Infect Dis 1997; 24: S213-21.
 12. Murray PR, Baron EJ, Pfaller MA, Tenoer FC, Tenover FC, Tenover FC. Manual of clinical microbiology. 7th ed. Washington D.C.: ASM, 1999.
 13. CLSI. Performance Standards for antimicrobial disk susceptibility tests; approved standard. 11th edition. M2-A11. Wayne, PA: CLSI, 2012.
 14. CLSI. Performance standards for antimicrobial susceptibility testing. 23rd informational supplement. M100-S23. Wayne, PA: CLSI, 2013.