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 $1.5x10^8$ 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 form 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% CO_2-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

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