

TMP 061 - MUELLER HINTON AGAR PLATE W/ 5% SHEEP BLOOD

INTENDED USE

For determination of susceptibility of *Streptococcus* species to antimicrobial agents.

PRODUCT SUMMARY AND EXPLANATION

Mueller Hinton agar was originally developed as a simple, transparent agar medium for cultivation of *Neisseria* species. It is further enriched by the addition of sterile blood. Mueller Hinton Agar is now used as a test medium for antimicrobial susceptibility testing. This media is used as a base for preparing media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives. Mueller Hinton Agar with 5% Sheep Blood is recommended for disc diffusion susceptibility testing of *Streptococcus pneumoniae* with selected agents.

The Kirby-Bauer procedure is based on agar diffusion of antimicrobial substances impregnated on paper discs. This method employs disc with a single concentration of antimicrobial agent and the zone diameters observed are correlated with minimum inhibitory concentration (MIC) values. A standardized suspension of the organism is swabbed over the entire surface of the medium. Paper discs impregnated with specific amounts of antimicrobial agents are then placed on the surface of the medium, incubated and zones of inhibition around each disc are measured. The susceptibility is determined by comparing with CLSI standards. The various factors, which influence disc diffusion susceptibility tests, are agar depth, disc potency, inoculum concentration, pH of the medium and beta-lactamase production by test organisms.

COMPOSITION

| Ingredients | Gms / Ltr |
|-------------------------|-----------|
| Beef, infusion from | 300.00 |
| Casein acid hydrolysate | 17.50 |
| Starch | 1.500 |
| Agar | 17.000 |
| Sheep blood | 50.00 ml |

PRINCIPLE

The medium consist of Beef extract and Casein acid hydrolysate which provides nitrogen, vitamins, carbon, and amino acids. Starch is added to absorb any toxic metabolites produced. Agar is the solidifying agent. The thymine/thymidine content of this medium is minimized (determined by disc diffusion procedure with *Enterococcus faecalis* ATCC 29212 and sulfamethoxazole-trimethoprim antibiotic) and levels of calcium and magnesium are adjusted (determined by *Pseudomonas aeruginosa* ATCC 27853 and aminoglycoside antibiotics) to give consistent zones of inhibition as per specified diameters in the CLSI standards. Sheep blood is added to enhance the growth of *Streptococcus pneumoniae*. The medium allows for better pigment production and more sharply defined haemolytic reactions.

INSTRUCTION FOR USE

Either streak, inoculate or surface spread the test inoculum aseptically on the plate.

QUALITY CONTROL SPECIFICATIONS

| | | |
|--------------------|---|--------------------------------|
| Appearance | : | Red colored medium |
| Quantity of Medium | : | 25ml of medium in 90mm plates. |
| pH (at 25°C) | : | 7.3± 0.2 |
| Sterility Check | : | Passes release criteria |



INTERPRETATION

Cultural response was observed after incubation with added 5% w/v sterile sheep blood.

| Microorganism | ATCC | Inoculum (CFU/ml) | Growth | Recovery | Haemolysis | Clindamycin (CD 2mcg) | Erythromycin (E 15mcg) | Vancomycin (VA 30 mcg) | Incubation Temperature | Incubation Period |
|---------------------------------|-------|-------------------|-----------|----------|------------|-----------------------|------------------------|------------------------|------------------------|-------------------|
| <i>Streptococcus pneumoniae</i> | 49619 | 50-100 | Luxuriant | >=70% | Alpha | 19-25 mm | 25-30 mm | 20-28 mm | 35-37°C | 18-24 Hours |
| <i>Streptococcus pyogenes</i> | 19615 | 50-100 | Luxuriant | >=70% | Beta | | | | 35-37°C | 18-24 Hours |

PACKAGING:

Doubled layered packing containing 5 No. of plates with one silica gel desiccant bag packed inside it.

STORAGE

On receipt, store the plates at 2-8 °C. Avoid freezing and overheating. Do not open until ready to use. Prepared plates stored in their original sleeve wrapping until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

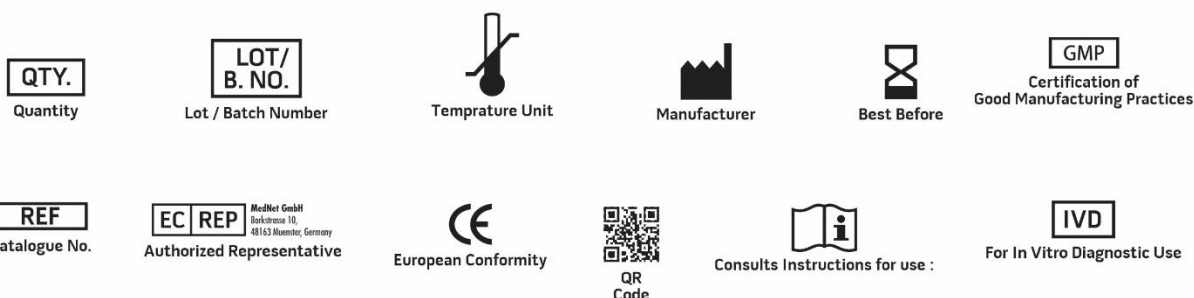
Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

- Bauer A. W., Kirby W. M., Sherris J. L. and Turck M., 1966, Am. J. Clin. Pathol., 45:493.
- Ericsson H. M. and Sherris J. L., 1971, Acta Pathol. Microbiol., Scand. Sect B Suppl., 217:1.
- Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
- Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (20 15) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
- Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Tenover F. C., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
- Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med.,48:330.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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