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TECHNICAL PRODUCT INFORMATION

MUELLER HINTON AGAR
Cat. No. P1460 (100 x 15 mm for 4 disks)
P2000 (150 x 15 mm for 12 disks)

MUELLER HINTON W/SHEEP BLOOD
Cat. No. P1470 (100 x 15 mm for 4 disks)
P2050 (150 x 15 mm for 12 disks)

INTENDED USE:

Mueller Hinton Agar is recommended for the antimicrobial disc diffusion susceptibility testing of common rapidly growing aerobic or facultative anaerobic microorganisms by the Kirby-Bauer method, [1-3] as standardized by the Clinical Laboratory Standards Institute[4] M2-A9, Ninth Edition (2006).

This product is not for use in human diagnostic procedures.

Mueller Hinton Agar w/5% Sheep Blood in recommended for antimicrobial disc diffusion susceptibility testing of *Streptococcus pneumoniae* with selected agents; i.e., chloramphenicol, erythromycin, ofloxacin, tetracycline and vancomycin in addition to Oxacillin screening for susceptibility to penicillin as standardized by CLSI.[4]

The addition of growth supplements may be added to Mueller Hinton Agar for testing of fastidious organisms such as *Haemophilus influenzae* or *Neisseria gonorrhoeae.*

HISTORY/SUMMARY:

Mueller Hinton medium was originally formulated by Mueller and Hinton as a protein free medium for the primary isolation of Neisseria species[1]. The medium became useful as a practical method of identifying sulfonamide resistant and sulfonamide responsive strains of gonococci[2]. However, with the development of modern antibiotics and the use of Thayer Martin medium for the culture of gonococci, neither of the above uses for Mueller Hinton medium remained effective. Thus, it became the recommended substrate for disc diffusion susceptibility as described by Bauer, Kirby, Sherris and Turck[1].

PRINCIPLES:

Mueller Hinton Agar is prepared from a dried infusion of beef, acid hydrolysate of casein to provide amino acids and other nitrogenous substances, minerals, vitamins, carbon and other nutrients to support growth of microorganisms. Soluble starch is added to absorb any toxic substances which may be present in the medium. The hydrolysis of the starch during autoclaving provides a small amount of dextrose as a source of energy. Agar is used as the solidifying agent. Mueller Hinton Agar is low in sulfonamide and tetracycline inhibitors and yields good growth with rapidly growing pathogens.

Organism sensitivity, (sensitive, intermediate, or resistant) is determined by comparing zone sizes obtained with the zone sizes listed in Standardized Zone Size Response Tables. Response Tables are supplied by Manufacturers of Antibiotic Diffusion Disks and are also included in the CLSI Document M100 (M2)[5].

FORMULA:

Ingredients per liter of DI Water:
Beef Extract..... 2.0 g
Starch..... 1.5 g
Casein, Acid hydrolysate..... 17.5 g
Agar..... 17.0 g

Supplemented with sources of tryptophan, cystine, biotin and dextrose as required for meeting performance criteria.
Sheep blood, Defibrinated ....50 mL
Final pH 7.3 ± 0.2 @ 25°C

PRECAUTIONS:
Since living organisms used with this material can be infectious to the user, proper handling and disposal methods should be established by the Laboratory director.

STORAGE:
Store the media at 2-8°C; do not use media after the expiration date. If, just before use, excess surface moisture is present the plates may be placed in an incubator (35°C) with lids cracked until the excess surface moisture is lost by evaporation (usually 10-30 minutes). The surface should be moist, but no droplets of moisture should be on the surface of the medium or on the petri dish covers when the plates are inoculated[5].

SPECIMEN COLLECTION:
Under the present practices, Mueller Hinton media is used for antimicrobial susceptibility testing and not as a medium for isolation of pathogens from clinical specimens.

PROCEDURE:
In the test procedure, a standardized suspension of the organism is swabbed over the entire surface of the medium. Paper discs impregnated with specific amounts of antibiotic or other antimicrobial agents are placed on the medium surface. The plate is incubated and the zones of inhibition around each disc are measured. Determination of susceptibility is made by comparing the zone size obtained to the zone sizes in CLSI Document M100(M2).[4]
NOTE:
Strict adherence to protocol is required to ensure reliable results. Numerous factors can affect results: inoculum size, rate of growth, medium formulation and pH, incubation time and environment, disc content and drug diffusion rate, and the measurements of the endpoints (zone size).

PERFORMANCE TEST:
Approval by NEL of each lot of Mueller Hinton is based, among other parameters, on the ability of this medium to support the growth of the organisms below described: with appropriate antibiotic zone sizes.

ORGANISMS		**RESULTS**	
		Media w/o Blood	Media w/5% Blood
Escherichia coli	ATCC# 25922	Good Growth	
Staphylococcus aureus	ATCC# 25923	Good Growth	Good Growth
Streptococcus faecalis	ATCC# 29212	Good Growth	Good Growth
Pseudomonas aeruginosa	ATCC# 27853	Good Growth	
Streptococcus pneumoniae	ATCC# 49619		Good Growth

DAILY QUALITY CONTROL:
CLIA' 88 Guidelines indicate the laboratory shall perform daily quality control testing of Mueller Hinton Agar, following the procedures and performance evaluation criteria stated in Clinical Laboratory Standards Institute[4] M2-A9, Ninth Edition (2006).[4]

REFERENCES:

1. Bauer, Kirby Sherris, and Turck, 1966 American Journal of Clinical Pathology 45:493
2. Ryan, Schoenknecht and Kirby, 1970 Hospital Practice 5:91
3. Mueller and Hinton Proc. Soc. Exp. Biol. Med., 48:330, 1941.
4. Clinical and Laboratory Standards Institute, 2006, Approved Standard: M2-A9 Performance standards for antimicrobial disk susceptibility tests, 9th edition, CLSI, Wayne, PA
5. Clinical and Laboratory Standards Institute, 2008, Performance Standards for antimicrobial susceptibility testing; eighteenth informational supplement , M100-S18(M2) CLSI, Wayne, PA

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