TM 236 - MUELLER HINTON AGAR NO.2

INTENDED USE

For testing susceptibility of common and rapidly growing bacteria using antimicrobial discs by using Kirby Bauer technique.

PRODUCT SUMMARY AND EXPLANATION

The goal of susceptibility test is to predict through an in vitro assessment the likelihood of successfully treating a patient's infection with a particular antimicrobial agent. The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* species. Other media were subsequently developed hat replaced the use of Mueller Hinton Agar for the cultivation of pathogenic *Neisseria* species, but it became widely used in the determination of sulfonamide resistance of gonococci and other organisms. Mueller Hinton Agar is now used as a test medium for antimicrobial susceptibility testing. Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in NCCLS (National Committee for Clinical Laboratory Standards), now CLSI (Clinical and Laboratory Standards Institute) Approved Standard. Mueller Hinton Agar has been selected by the CLSI for several reasons: i.e. It demonstrates good batch-to-batch reproducibility for susceptible testing. ii. It is low in sulfonamide, trimethoprim and tetracycline inhibitors. iii. It supports the growth of most non-fastidious bacterial pathogens and iv. Many data and much experience regarding its performance have been recorded. Mueller Hinton Agar No. 2 is used in the susceptibility testing of rapidly growing aerobic and facultative anaerobic bacteria from clinical specimens. Kirby-Bauer et al recommended this medium for performing antibiotic susceptibility tests using a single disc of high concentration. WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility. The medium is designed to give a low thymine and thymidine content and also the calcium and magnesium ion concentration is adjusted as recommended by CLSI. The medium is not recommended for fastidious organisms. Thymine and thymidine inhibit sulfonamide and trimethoprim activity and calcium and magnesium interferes with the activity of aminoglycoside antibiotics.

COMPOSITION

Ingredients	Gms / Ltr
Beef heart infusion	2.000
Casein acid hydrolysate	17.500
Starch	1.500
Agar	17.000

PRINCIPLE

Beef heart infusion and Casein acid hydrolysate provide nitrogenous compounds, carbon, sulphur and other essential nutrients. Starch acts as a protective colloid against toxic substances present in the medium. Starch hydrolysis yields dextrose, which serves as a source of energy. These ingredients are selected for low thymine and thymidine content as determined by MIC values for *Enterococcus faecalis* with sulfamethoxazole trimethoprim. Calcium and magnesium ion concentrations are adjusted to provide the amounts recommended by CLSI to give the correct MIC values with aminoglycosides and *Pseudomonas aeruginosa.* 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 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.

INSTRUCTION FOR USE

- Dissolve 38.0 grams in 1000 ml purified / distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- Cool to 45-50°C. Mix well and pour into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium : Light amber coloured clear to slightly opalescent gel forms in Petri plates.
pH (at 25°C) : 7.3±0.1

INTERPRETATION

Cultural characteristics observed after an incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Incubation Temperature	Incubation Period
Escherichia coli	25922	50-100	Luxuriant	>=70%	35-37°C	18-24 Hours
Haemophilus influenzae	49247	50-100	Good-luxuriant (on Mueller Hinton Chocolate Agar)	>=70%	35-37°C	18-24 Hours
Neisseria gonorrhoeae	49226	50-100	Luxuriant	>=70%	35-37°C	18-24 Hours
Pseudomonas aeruginosa	27853	50-100	Luxuriant	>=70%	35-37°C	18-24 Hours
Staphylococcus aureus subsp. aureus	25923	50-100	Luxuriant	>=70%	35-37°C	18-24 Hours
Enterococcus faecalis	29212	50-100	Luxuriant	>=70%	35-37°C	18-24 Hours
Streptococcus pneumoniae	6305	50-100	Luxuriant (on Mueller Hinton Blood Agar)	>=70%	35-37°C	18-24 Hours

PACKAGING:

In pack size of 100 gm and 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

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

DISPOSAL

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

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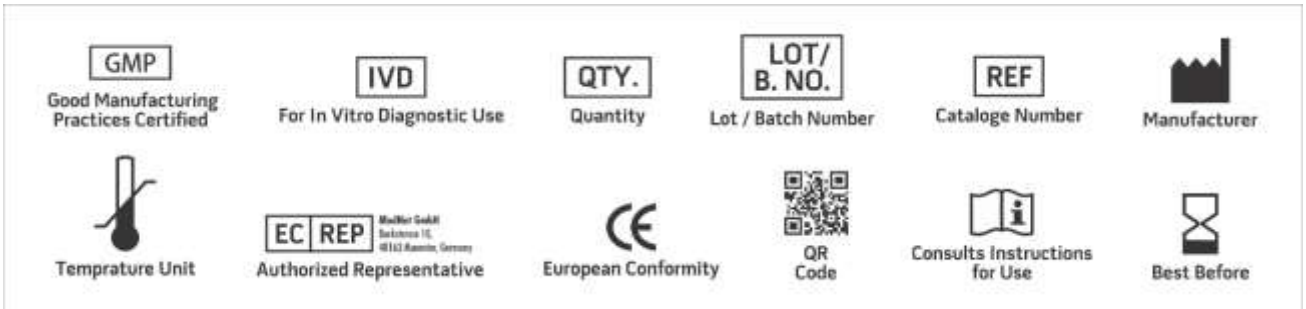

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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