TM 2250 - MUELLER HINTON AGAR 2% GLUCOSE W/ METHYLENE BLUE

INTENDED USE

Mueller Hinton Agar, Modified (as per CLSI for antifungal) is recommended for testing performing Antifungal Disk Diffusion Susceptibility of yeasts.

SUMMARY AND EXPLANATION

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic species. Mueller Hinton Agar, Modified (as per CLSI for antifungal) is recommended for the diffusion of antifungal agents impregnated on paper disc through an agar gel as described in CLSI Approved Standard. When supplemented with glucose to a final concentration of 2%, it provides for suitable fungal growth. The addition of methylene blue to a final concentration of 5µg/ml enhances zone edge definition. Kirby-Bauer et al recommended Mueller Hinton Agar 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. Mueller Hinton Agar with 5% sheep blood and Mueller Hinton Agar with Haemoglobin have been recommended for antimicrobial susceptibility testing of *Streptococcus pneumoniae* and *Haemophilus influenzae*. Similarly Mueller Hinton Agar, Modified (as per CLSI for antifungal) is recommended for antifungal susceptibity testing of discs.

COMPOSITION

Ingredients	Gms / Ltr
Dextrose (Glucose)	20.000
casein acid hydrolysate	17.500
Agar	17.000
Beef infusion	2.000
Starch	1.500
Methylene blue	0.0005

PRINCIPLE

Beef 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. Dextrose (Glucose) serves as an energy source for fungal cultures while Methylene blue enhances zone edge definition.

INSTRUCTION FOR USE

- Dissolve 58 grams in 1000 ml 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 : Light yellow to yellow may have slight blue tinge homogeneous free flowing powder.
Appearance of prepared medium : Amber coloured clear to slightly opalescent gel forms in Petri plates.
pH (at 25°C) : 7.3 ± 0.1

INTERPRETATION

A luxuriant growth of test organisms was observed on Mueller Hinton Agar, Modified (as per CLSI for antifungal). Along with inhibition zones with respective antibiotic concentrations.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Amphotericin B AP(100units)	Amphotericin B AP(20 mcg)	Amphotericin B AP(50 mcg)	Incubation Temperature	Incubation Period
Candida albicans	90028	10 - 100	Luxuriant	>=70%	10 -17 mm	10 -15 mm	31- 42 mm	33-37°C	24-48 Hours
Candida parapsilosis	22019	10 - 100	Luxuriant	>=70%	11 -20 mm	10 -17 mm	28 -37 mm	33-37°C	24-48 Hours
Candida tropicalis	750	10 - 100	Luxuriant	>=70%	8 -12 mm	8 -10 mm	13 -17 mm	33-37°C	24-48 Hours
Candida krusei	6258	10 - 100	Luxuriant	>=70%	9 -14 mm	8 -12 mm	16 -25 mm	33-37°C	24-48 Hours
Candida albicans	10231	10 - 100	Luxuriant	>=70%	10 -18 mm	10 -16 mm	30 -40 mm	33-37°C	24-48 Hours
Saccharomyces cerevisiae	9763	10 - 100	Luxuriant	>=70%	11 -18 mm	8 -12 mm	29 -38 mm	33-37°C	24-48 Hours

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°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.

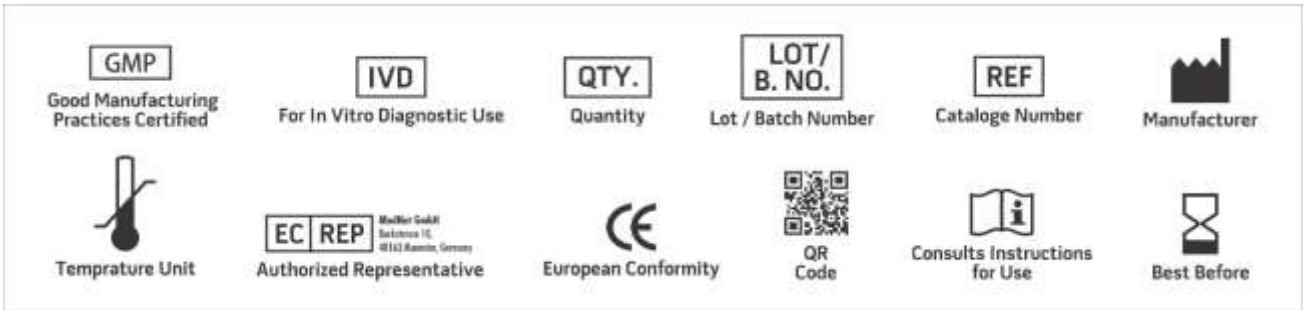
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

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

REFERENCES

1. Present Status and Future Work, WHO Sponsored collaborative study, Chicago, Oct. 1967.
2. Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med., 48:330
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Method for Antifungal Disk Diffusion Susceptibilty Testing of yeasts; Approved Guideline Second Edition M44-A2 Vol.24 No.17.
6. Bauer A. W., Kirby W. M., Sherris J. L. and Turck M., 1966, Am. J. Clin. Pathol., 45:493.



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

***For Lab Use Only**

Revision: 08 Nov., 2019