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ISO 9001-2008 Certified Company

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TECHNICAL DATA SHEET

Mueller Hinton Agar 2% Glucose W/Methylene Blue

Principle

Mueller Hinton agar with 2% glucose and methylene blue is the modification of formula recommended by Mueller and Hinton (1941) for the primary isolation of *Neisseria species*. Commonly it is used for performing antibiotic susceptibility tests using a disc diffusion assay and due to its susceptibility for microorganism and high reproducibility; WHO Committee recommended it for standardization of susceptibility testing. Media is simple and composed of meat infusion form (equivalent of beef infusion form), Casamino acids, starch, glucose, methylene blue and agar. Meat Infusion from and casamino acids provides nitrogen vitamins, amino acids, minerals, carbon and other nutrients to support the growth of microorganisms. While starch absorb any toxic metabolites produced during microbial growth and while autoclaving undergoes hydrolysis and liberate small amount of dextrose, which act as source of energy. The additional glucose serves as an energy source for fungal cultures while Methylene blue enhances zone edge definition. Agar is the solidifying agent.

Use: For testing performing Antifungal Disk Diffusion Susceptibility of yeasts

Contents*

Ingredients	Gram/Litre
Meat Infusion Form#	300.000
Casamino Acids	17.500
Starch	1.500
Glucose	20.000
Methylene blue	0.0005
Agar	17.000
pH at 25˚C	7.3 ±0.1

*** Formula adjusted for optimum performance and parameters**
 # Equivalent to Beef infusion form

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Directions: Dissolve 58.00 grams in 1000 ml distilled water. Boil to dissolve the medium completely and sterilize by autoclaving at 15 lbs. pressure (121 °C) for 15 min, cool it to 42-45 °C and distribute aseptically in petri plates. Ensure complete solidification and inoculate test sample aseptically.

Specimens types analyzed

Pharmaceutical samples, clinical and non-clinical samples etc.

Precautions to be taken

These microbial media are intended for the in-vitro use only. All the handling, experiments, storage, and discarding should be performed with the help of skilled and knowledgeable technicians and as per the established guidelines. The material should be disposed only after proper sterilization by autoclaving. Please go through the MSDS of the media to avoid any accidents or in emergency.

Performance and Evaluation

The expected performance of the medium is liable to use as per the direction on the label when stored at optimum conditions and within expiry date.

Quality Control

Appearance	**Light yellow with slight bluish tinge, homogeneous powder**
Reaction of 5.8% solution	**7.3 ±0.1 at 25 ˚C**
pH	**7.20- 7.40**
Gelling	**Firm comparable with 1.7% agar gel**
Color and clarity of ready medium	**Light yellow colored clear to slightly opalescent gel**
Growth Promotion properties	**Best at ≤ 100 CFU at 32-37 ºC for 18-72 h**
Indicative properties	**Optimum at ≤ 100 CFU at 32-37 ºC for 18-48 h**
Negative control	**Performed using sterile distilled water**

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Different Microbial Response

The growth performance of organisms was observed on modified Mueller Hinton Agar in 24-48 hours at 33-37°C along with inhibition zones with respective antibiotic concentrations.

Organism	ATCC	Inoculum	Growth	Recovery
Candida albicans	10231	50-100	Luxuriant	≥ 70%
Saccharomyces cerevisiae	9763	50-100	Luxuriant	≥ 70%

Antibiotic Sensitivity test
Amphotericin B 10 mcg and 5 0 mcg discs w ere tested for standard ATCC strains and zone of inhibition were measured after an incubat ion 33-37°C for 24-48 hours.

Organism	ATCC	Growth	Zone of inhibition Amphotericin B	
			(10 mcg)	(50 mcg)
Candida albicans	10231	Luxuriant	10-17 mm	31-42 mm
Saccharomyces cerevisiae	9763	Luxuriant	11-18 mm	29-38 mm

Storage and Shelf Life: The product is highly hygroscopic; keep the container tightly closed at all times and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Note: Sterilize media immediately after reconstitution.

Disposal: To avoid the contamination or propagation of any hazardous microbes the used, unusable or modified preparation of this product must be disposed after autoclaving after completion of task.

Reference

1. **Bauer, A. L., W. M. M. Kirby, J. C. Sherris, and M. Turck. (1966).** ***Antibiotic susceptibility testing***
2. **Mueller, J. H., and J. Hinton (1941).** ***A protein-free medium for primary isolation of gonococcus and meningococcus.*** **Proc. Soc. Exp. Biol. Med. 48:330-333.**

Method for Antifungal Disk Diffusion Susceptibility Testing of yeasts; Approved Guideline Second Edition M44-A2 Vol.24 No.17.

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