Technical Data

Mueller Hinton AgarPlate 2% Glucose w/ Methylene blue MP1825

Intended Use:

Recommended for performing Antifungal Disk diffusion Susceptibilty Testing of yeasts.

Composition**

Ingredients	g / L
HM infusion B #	2.000
Acicase™	17.500
Starch	1.500
Dextrose (Glucose)	20.000
Methylene blue	0.0005
Agar	17.000
Final pH (at 25°C)	7.3±0.1

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef Infusion from

Directions

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

Principle And Interpretation

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic species (1). 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 (2). 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 (3). WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility (4). 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.

HM infusion B and Acicase™ 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.

Type of specimen

Isolated Microorganism from clinical samples.

Specimen Collection and Handling

Technique:

Preparation of Inoculum:

1. Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (MP063) and incubated at 35 ± 2°C. Colonies are suspended in 5ml of sterile 0.85% Saline.
2. Vortex the resulting suspension and adjust the turbidity to yield 1 x 10^6 - 5 x 10^6 cells /ml (i.e. 0.5 McFarland standard).

Test Procedure:

1. Prepared plates of Mueller Hinton Agar 2% Glucose w/ Methylene blue (as per CLSI for antifungal) is used for carrying out susceptibility of antifungal discs.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the Petri plate) and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.

3. Apply the discs using aseptic technique. Deposit the discs with centers at least 24 mm apart.
4. Invert the plates and place in an incubator set to 35 ± 2°C within 15 minutes after the discs are applied.
5. Examine each plate after 20 - 24 hours of incubation. If plate was satisfactorily streaked the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. Read at 48 hours only when insufficient growth is observed after 24 hours incubation.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use. For professional use only. Read the label before opening the pack. Wear protective gloves/ protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. This medium is recommended for susceptibility testing of pure cultures only.
2. Inoculum density may affect the zone size. Heavy inoculum may result in smaller zones or too less inoculum may result in bigger zones.
3. Fastidious organisms may not grow on this medium due to nutritional variations.
4. Antifungal disc are used to carry out the susceptibility, proper storage of the disc is desired which may affect the potency of the disc.
5. Under certain circumstances, the in vitro results of antifungal susceptibility may not show the same in vivo.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Sterile Mueller Hinton Agar with 2% Glucose and Methylene blue in 90 mm disposable plates with smooth surface & absence of black particles/ cracks/ bubbles.

Colour of the medium

Light green coloured medium

Quantity of medium

25 ml of medium in 90mm disposable plates.

pH

7.20-7.40

Cultural response

Various discs were tested for standard ATCC strains and zone of inhibition were measured after an incubation 30-35°C for 24-48 hours. (As per the latest CLSI Protocol M6 & Standards as per the current CLSI M100) (# Concentrations of Antibiotics are as per CLSI standards)

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotic	Standard Zone
***Candida albicans* ATCC 90028 Growth promoting**	50-100	Luxuriant	>=70%	Amphotericin-B AP 100 units	10-17 mm
				Amphotericin-B AP 50 mcg	12-15 mm
				Amphotericin-B AP 20 mcg	10-15 mm
				Clotrimazole CC 10 mcg	18-32 mm
				Fluconazole FLC 25 mcg#	28-39 mm
				Fluconazole FLC 10 mcg	27-38 mm
				Itraconazole IT 30 mcg	18-22 mm
				Itraconazole IT 10 mcg	16-20 mm
				Ketoconazole KT 50 mcg	37-45 mm
				Ketoconazole KT 30 mcg	32-36 mm
				Ketoconazole KT 10 mcg	20-32 mm
				Miconazole MIC 50 mcg	26-32 mm
				Miconazole MIC 30 mcg	22-26 mm

				Nystatin NS 100 units	19-27 mm
				Nystatin NS 50 mcg	19-23 mm
				Voriconazole VRC 1 mcg#	31-42 mm
***Candida parapsilosis* ATCC 22019 Growth promoting**	50-100	Luxuriant	>=70%	Amphotericin-B AP 100 units Amphotericin-B AP 50 mcg Amphotericin-B AP 20 mcg Clotrimazole CC 10 mcg Fluconazole FLC 25 mcg# Fluconazole FLC 10 mcg Itraconazole IT 30 mcg Itraconazole IT 10 mcg Ketoconazole KT 50 mcg Ketoconazole KT 30 mcg Ketoconazole KT 10 mcg Miconazole MIC 50 mcg Miconazole MIC 30 mcg Nystatin NS 100 units Nystatin NS 50 mcg Voriconazole VRC 1 mcg#	11-20 mm 13-17 mm 10-17 mm 16-30 mm 22-33 mm 22-33 mm 20-24 mm 11-18 mm 36-44 mm 26-32 mm 14-29 mm 23-29 mm 13-17 mm 16-25 mm 19-23 mm 28-37 mm
***Candida tropicalis* ATCC 750 Growth promoting**	50-100	Luxuriant	>=70%	Amphotericin-B AP 100 units Amphotericin-B AP 50 mcg Amphotericin-B AP 20 mcg Clotrimazole CC 10 mcg Fluconazole FLC 25 mcg# Fluconazole FLC 10 mcg Itraconazole IT 30 mcg Itraconazole IT 10 mcg Ketoconazole KT 50 mcg Ketoconazole KT 30 mcg Ketoconazole KT 10 mcg Miconazole MIC 50 mcg Miconazole MIC 30 mcg Nystatin NS 100 units Nystatin NS 50 mcg	8-12 mm 13-17 mm 8-10 mm 10-20 mm 26-37 mm 16-25 mm 11-18 mm 8-13 mm 27-34 mm 26-32 mm 17-28 mm 14-20 mm 14-20 mm 16-21 mm 13-17 mm
***Candida krusei* ATCC 6258 Growth promoting**	50-100	Luxuriant	>=70%	Amphotericin-B AP 100 units Amphotericin-B AP 50 mcg Amphotericin-B AP 20 mcg Clotrimazole CC 10 mcg Itraconazole IT 30 mcg Itraconazole IT 10 mcg Ketoconazole KT 50 mcg Ketoconazole KT 30 mcg Ketoconazole KT 10 mcg	9-14 mm 14-20 mm 8-12 mm 14-24 mm 8-15 mm 8-15 mm 19-26 mm 19-26 mm 10-14 mm

				Miconazole MIC 50 mcg 19-26 mm Miconazole MIC 30 mcg 19-26 mm Nystatin NS 100 units 15-20 mm Nystatin NS 50 mcg 19-26 mm Voriconazole VRC 1 mcg# 16-25 mm
***Candida albicans* ATCC 10231 (00054*)**				
Growth promoting	50-100	Luxuriant	>=70%	Amphotericin-B AP 100 10-18 mm units Amphotericin-B AP 50 15-23 mm mcg Amphotericin-B AP 20 10-16 mm mcg Clotrimazole CC 10 mcg 12-18 mm Fluconazole FLC 10 mcg 18-22 mm Itraconazole IT 30 mcg 18-22 mm Itraconazole IT 10 mcg 18-22 mm Ketoconazole KT 50 mcg 31-40 mm Ketoconazole KT 30 mcg 31-40 mm Ketoconazole KT 10 mcg 18-22 mm Miconazole MIC 50 mcg 20-27 mm Miconazole MIC 30 mcg 20-27 mm Nystatin NS 100 units 15-23 mm Nystatin NS 50 mcg 16-25 mm
***Saccharomyces cerevisiae* ATCC 9763 (00058*)**				
Growth promoting	50-100	Luxuriant	>=70%	Amphotericin-B AP 100 units 11-18 mm Amphotericin-B AP 50 mcg 16-25 mm Amphotericin-B AP 20 mcg 8-12 mm Clotrimazole CC 10 mcg 17-25 mm Miconazole MIC 50 mcg 20-28 mm Miconazole MIC 30 mcg 20-28 mm Nystatin NS 100 units 17-25 mm Nystatin NS 50 mcg 22-27 mm

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

On receipt store between 20-30°C. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

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

Revision : 02/2024

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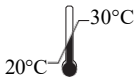
IVD ***In vitro*** **diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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www.cepartner4u.eu

CE Marking

Do not use if package is damaged

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