HiCrome™ Mueller Hinton Agar (For Antifungal testing)

Recommended for the chromogenic differentiation of yeasts from clinical samples and determination of susceptibility to antifungal agents.

M2067

Composition **

Ingredients	Grams/Litre
Acicase #	14.00
Dextrose (Glucose)	20.00
Chromogenic mixture	1.80
Agar	17.00
Final pH (at 25°C)	7.3 ± 0.1

** Formula adjusted, standardized to suit performance parameters
#Equivalent to Casein acid hydrolysate

Directions

Suspend 52.8 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates. The performance of this batch has been tested and standardized as per the current CLSI (formerly NCCLS) document M44-A2 in Method for Antifungal Disk Diffusion susceptibilty Testing of yeasts.

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. Kirby-Bauer et al recommended Mueller Hinton Agar for performing antibiotic susceptibility tests using a single disc of high concentration (4). WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility (3). 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. Perry and Miller (1) reported that *Candida albicans* produces an enzyme β -N-acetyl- galactosaminidase and according to Rousselle et al (2) incorporation of chromogenic or fluorogenic hexosaminidase substrates into the growth medium helps in identification of C.albicans isolates directly on primary isolation.

Acicase provide nitrogenous and carbonaceous compounds, long chain amino acids, 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.

Glucose serves as an energy source for fungal cultures. *C.albicans* appear as light green coloured smooth colonies, *C.tropicalis* appear as blue to metallic blue coloured raised colonies. *C.glabrata* colonies appear as cream to white smooth colonies, while *C.krusei* appear as purple fuzzy colonies.

Type of specimen

Clinical samples : Pure cultures isolated from urine , stool, blood etc.

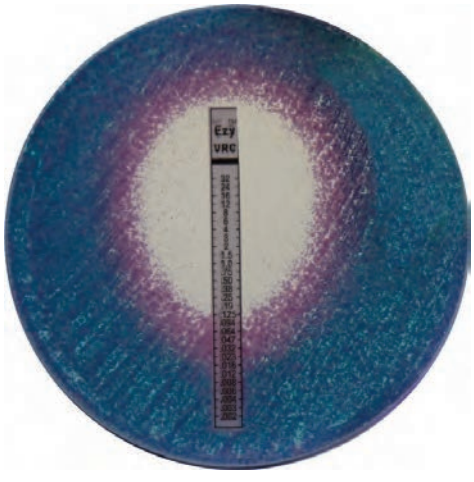
Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5, 6).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. 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.



M2067 HiCrome™ Mueller Hinton Agar (For Antifungal testing) Mixture

HiCromeVeg™ Single Streak Rapid Differentiation Series *Freedom from BSE / TSE worries*

HiCrome™ *Candida* Differential Agar / Base, Modified (M1297A)/(M1456A) is also available as HiCrome™ *Candida* Differential HiVeg™ Agar / Base, Modified (MV1297A)/(MV1456A) / HiCrome™ *Candida* Differential HiCynth™ Agar Base (MCD1297A) wherein all the animal origin nutrients have been replaced by vegetable based nutrients & Chemically defined peptones respectively.

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Limitations

1. This medium is recommended for susceptibility testing of pure cultures only.
2. Inoculum density may effect 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.
4. Certain species of Candida may show variation in colour intensity depending on the presence of enzyme.
5. As antimicrobial susceptibility is carried with antibiotic disc, proper storage of the disc is desired which may effect the potency of the disc. Under certain circumstances, the in vitro results of antibiotic 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 recommended temperature.

Quality Control

Appearance of Powder	:	Cream to yellow homogeneous free flowing powder.
Gelling	:	Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium	:	Yellow to amber coloured opalescent gel forms in Petri plates
Reaction	:	Reaction of 5.28% w/v aqueous solution at 25°C. pH : 7.3±0.2
Cultural Response	:	A luxuriant growth of test organisms was observed in 48 hours at 33-37°C along with inhibition zones with respective antibiotic concentrations.

Organisms (ATCC)	Growth	Colour of the colony	Sensitivity testing	
			Fluconazole FLC (25 mcg)	Voriconazole VRC (1 mcg)
Candida albicans (90028)	luxuriant	light green	28-39 mm	31-42mm
Candida parapsilosis 22019	luxuriant	cream	22-33 mm	28-37 mm
Candida tropicalis (750)	luxuriant	blue to purple	26-37mm	-
Candida krusei (6258)	luxuriant	purple	-	16-25mm

Storage and Shelf-life

Store between 2-8°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. 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).

References

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.
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4. Bauer A. W., Kirby W. M., Sherris J. L. and Turck M., 1966, Am. J. Clin. Pathol., 45:493.
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