HiCrome™ Mueller Hinton Agar

Recommended for differentiation of organisms based on chromogenic differentiation and determination of susceptibility of microorganisms to antimicrobial agents.

M2010

Composition **

Ingredients	Grams/Litre
Acicase#	20.00
Chromogenic mixture	1.50
Agar	17.00

Final pH (at 25°C) 7.3±0.1
** Formula adjusted, standardized to suit performance parameters
# Casein acid hydrolysate

Directions

Suspend 38.50 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

The Mueller Hinton formulation was originally developed for the cultivation of pathogenic *Neisseria* species (1). Other media were subsequently developed that 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 (2).

Acicase provide nitrogenous compounds, carbon, sulphur and other essential nutrients. These ingredients are selected for low thymine and thymidine content as determined by MIC values for *Enterococcus faecalis* with sulfamethoxazole trimethoprim (SXT). Chromogenic mixture incorporated helps in colour differentiation. One of the chromogenic substrate is cleaved by β-glucosidase possessed by Enterococci resulting in formation of blue colonies. *E.coli* produce pink to purple colonies due to the enzyme β-D-galactosidase that cleaves the other chromogenic substrate. *Staphylococcus aureus* produces colourless colonies. *Pseudomonas aeruginosa* produces greenish pigmentation. *Klebsiella* and *Enterobacter* species produces metallic blue colured colonies. Colonies of *Proteus*, *Morganella* and *Providencia* species appear brown .This medium can be employed in screening urinary tract pathogens wherein organisms can be differentiated based on colour and simultaneously the antibiotic sensitivity can be determined.

Type of specimen

Clincal samples

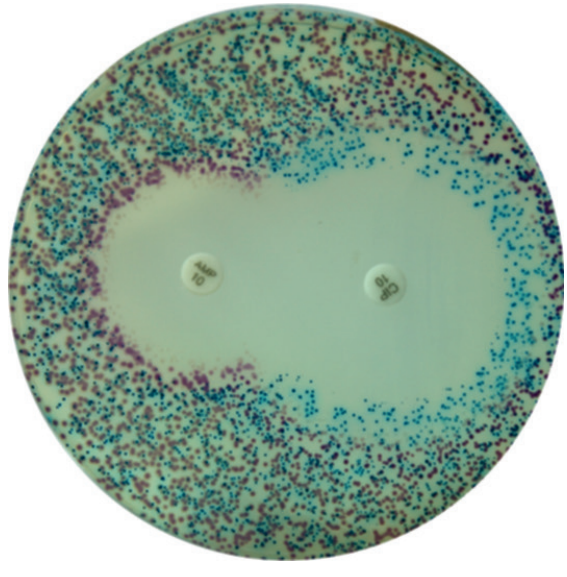
Specimen Collection and Handling

For clinical samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3,4).

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



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Mixture of ***Klebsiella***, *S. faecalis* and ***E. coli***

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Limitations

1. Inoculum density may effect the zone size. Heavy inoculum may result in smaller zones or too less inoculum may result in bigger zones.
2. As antimicrobial susceptibility is carried with antibiotic disc, proper storage of the disc is desired which may effect the potency of the disc.
3. 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 1.7% agar gel.

Colour and Clarity : Light amber coloured clear to slightly opalescent gel froms in Petri plates

Reaction : Reaction of 3.85% w/v aqueous solution at 25°C. pH : 7.3±0.1

Cultural Response : Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli (25922) (00013*)	50-100	luxuriant	≥70%	pink-purple
Pseudomonas aeruginosa (27853) (00025*)	50-100	luxuriant	≥70%	greenish pigment may be observed
Staphylococcus aureus (25923) (00034*)	50-100	luxuriant	≥70%	colour-less-golden yellow
Enterococcus faecalis (29212) (00087*)	50-100	luxuriant	≥70%	blue
Klebsiella pneumoniae (13883) (00097*)	50-100	luxuriant	≥70%	metallic blue

Key : * = corresponding WDCM Numbers

Storage and Shelf-life

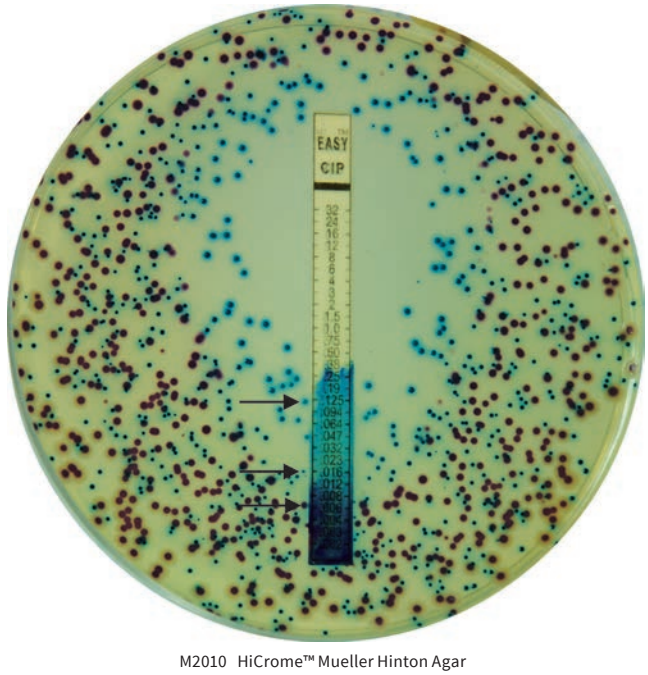
Store between 2-8°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage 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 (3,4).

References

1. Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med., 48:330.
2. National Committee for Clinical Laboratory Standards, 2000, Approved Standard: M7-A5. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that grow aerobically, 5th Ed., NCCLS, Wayne, Pa.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. 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.



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