Mueller Hinton HiVeg® Broth

MV391

Intended Use:

Recommended for determination of Invitro susceptibility of bacterial strains against antibacterial agents by broth dilution methods.

Composition**

Ingredients	g/ L
HiVeg® infusion from 300 g	2.000
HiVeg® acid hydrolysate	17.500
Starch	1.500
Final pH (at 25°C)	7.3±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 21.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Mix well and dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Note : It is suggested to boil the medium before autoclaving to avoid settling of starch at the bottom.

Principle And Interpretation

Mueller Hinton Broth is a stable serum-free bacteriological media that supported the growth of two otherwise very fastidious bacteria, the *meningococcus* and the *gonococcus* (1). Later it was improved by supplementing cations to enhance *Pseudomonas* recovery and for propagating the vast majority of pathogenic bacteria encountered in clinical practice. In the beginning of the antibiotic era, the versatility of Mueller Hinton Broth and its agar was a perfect match, allowing a standardized, consistent medium for broth dilution or disk diffusion testing of antibiotic potency, defined most frequently in terms of minimum inhibitory concentration (MIC) (2). In recent decades, MHB has been endorsed by the Clinical and Laboratory Standards Institute (CLSI), the global nonprofit organization that ensures quality in healthcare testing, as the appropriate media for routine bacterial antibiotic susceptibility determination, with updated cutoff standards for designating resistant (R) and susceptible (S) strains (CLSI) (3).Mueller Hinton HiVeg® Broth is prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks.

HiVeg® infusion and HiVeg® acid hydrolysate provide nitrogenous compounds, carbon, sulphur and other essential nutrients. Starch acts as a protective colloid against toxic substances present in the medium. These ingredients are selected for low thymine and thymidine content as determined by MIC values for *Enterococcus faecalis* with sulfamethoxazole trimethoprim (SXT). The CLSI recommend the following cation levels Ca++, 20-25mg/liter; Mg++, 10-12.5mg/liter to be present in the broth while performing broth dilution method (1). Cation level is usually inadequate in the medium, the concentration of cations already present in the broth must be accounted for calculating the amount of Ca++ or Mg++; if that needs to be added. Cation stock for addition of supplemental cations are recommended by CLSI which is 8.36g of $MgCl_2.6H_2O$ in 100ml contains 10mg of Mg++/ml and 3.68g of $CaCl_2.2H_2O$ in 100ml contains 10 mg of Ca++/ml. Alternatively Mueller Hinton Broth No. 2 Control Cations (M1657) is available wherein cations are adjusted as per Standard guidelines.

Type of specimen

Isolated microorganism

Specimen Collection and Handling:

For samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

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

Warning and Precautions :

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3.This medium is recommended for susceptibility testing of pure cultures only.
4.Under certain circumstances, the in vitro results of antibiotic susceptibility may not show the same in vivo.
5.pH of the medium affects susceptibility of Aminoglycosides, Tetracyclines and Quinolones.
6.Aminoglycosides and Tetracyclines are affected by Ca++ and Mg++ content.

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

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light amber coloured clear solution in tubes

Reaction

Reaction of 2.1% w/v aqueous solution at 25°C. pH : 7.3±0.1

pH

7.20-7.40

Cultural Response

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

Organism	Inoculum (CFU)	Growth
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant
Pseudomonas aeruginosa ATCC 27853(00025*)	50-100	good-luxuriant
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	good-luxuriant
Enterococcus faecalis ATCC 19433 (00009*)	50-100	good-luxuriant

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order 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. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. Mueller J.H., Hinton J. A protein-free medium for primary isolation of the *Gonococcus* and *Meningococcus*. Proc. Soc. Exp. Biol. Med. 1941;48:330–333.
2. Victor Nizet; "The Accidental Orthodoxy of Drs. Mueller and Hintion"; EBiomedicine 22 (2017) 26-27.PMID: 28689739.
3. (CLSI): Clinical and Laboratory Standards Institute. 25th Informational Supplement. Clinical and Laboratory Standards Institute; Wayne, PA: 2015. Performance standards for antimicrobial susceptibility testing; p. M100-S25.

4..Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. 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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IVD ***In vitro*** **diagnostic medical device**

30°C
10°C **Storage temperature**

EU REP AR Experts BV
Boeingavenue 209
1119 PD Schiphol-Rijk
The Netherlands

CE Marking

Do not use if package is damaged

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