Columbia C.N.A. HiVeg™ Agar Base w/ 1% Agar

MV560A

Intended Use:

Recommended for selective isolation of pathogenic gram-positive cocci from various specimens.

Composition**

Ingredients	g / L
HiVeg™ peptone No. 5	20.000
HiVeg™ infusion	3.000
Corn starch	1.000
Sodium chloride	5.000
Colistin sulphate	0.010
Nalidixic acid	0.015
Agar	10.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 39.02 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 5% v/ v sterile, defibrinated blood. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Columbia Agar Base is a nutritionally rich formula containing 5% defibrinated blood, which provides more nutrients and capability of displaying haemolytic reactions. Columbia Blood Agar Base is utilized as a base for preparation of media containing blood and in selective media preparations where various combinations of antimicrobial agents are used as additives. Ellner et al formulated the medium (1) and found that the combination of peptones used gave more rapid and abundant growth of Streptococci, Staphylococci, *Neisseria* and *Haemophilus* with better-defined haemolytic reactions. Columbia C.N.A. HiVeg™ Agar Base w/ 1% Agar is prepared with the same formula as Columbia Agar Base with the addition of 10 mg/litre of colistin and 15 mg/litre of nalidixic acid to inhibit the growth of gram-negative bacteria and to support the growth of Staphylococci, haemolytic Streptococci and Enterococci when supplemented with 5% blood. Columbia C.N.A. HiVeg™ Agar Base w/ 1% Agar is prepared by completely replacing animal based peptone with vegetable peptones to avoid BSE/TSE risks associate with animal peptones. HiVeg™ peptone No. 5 and HiVeg™ infusion supports luxuriant growth of microorganisms and visualization of good haemolytic reactions. Sheep blood allows detection of haemolytic reactions and supplies X-factor necessary for the growth of many bacterial species. Horse blood supplies X-factor and V-factor, therefore is mostly preferred in most laboratories. Yeast extract and cornstarch serve as energy source and neutralizer respectively.

It should be noted that this medium has relatively high carbohydrate content and, therefore, beta-hemolytic streptococci may produce a greenish hemolytic reaction that may be mistaken for alpha haemolysis. The addition of the antimicrobial agents, colistin (or polymyxin B) and nalidixic acid, renders the medium selective for gram-positive microorganisms (2). Colistin and nalidixic acid disrupt the cell membrane of gram-negative organisms, whereas nalidixic acid blocks DNA replication in susceptible gram-negative bacteria (3).

Columbia C.N.A. HiVeg™ Agar Base w/ 1% Agarwith addition of blood gives selective isolation of gram-positive cocci, Staphylococci and Streptococci, particularly when gram-negative bacilli are present and tend to overgrow on conventional blood agar plates. Also used for selective isolation of *Gardnerella vaginalis*. This medium supports growth of *Brucella abortus, Yersinina pestis, Clostridium perfringens* and all commonly occurring *Enterobacteriaceae* without addition of blood.

Type of specimen

Dairy samples; Water samples

Specimen Collection and Handling:

For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (4,5). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(6) 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. Further biochemical and serological tests must be carried out for further identification.

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

Gelling

Firm, comparable with 1.0% Agar gel

Colour and Clarity of prepared medium

Basal medium: Yellow ;After addition of 5% v/v sterile defibrinated blood: Cherry red coloured Basal medium:Clear to slightly opalescent ; After Addition: opaque gel forms in Petri plates

Reaction

Reaction of 4.4% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed with added 5% v/v sterile, defibrinated blood after an incubation at 35-37°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Neisseria meningitidis ATCC 13090	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	luxuriant	>=50%	beta/gamma
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	luxuriant	>=50%	gamma
Streptococcus pneumoniae ATCC 6303	50-100	luxuriant	>=50%	alpha
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	>=50%	beta

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store dehydrated 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 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 (5,6).

Reference

1. Ellner et al, 1966, Am. J. Clin. Path., 45:502.
2. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
3. Estevez, 1984, Lab. Med., 15:258.
4. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
5. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
6. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
8. 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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