Dehydrated Culture Media
Bases / Media Supplements

Technical Information

Wilkins Chalgren Anaerobic Agar Base

Product Code: DM 1832

Application: - Wilkins Chalgren Anaerobic Agar Base is used for the isolation and cultivation of anaerobic bacteria and also for susceptibility testing of anaerobic bacteria by the agar dilution method.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Peptic digest of animal tissue	10.000
Yeast extract	5.000
Dextrose	1.000
Sodium chloride	5.000
L-Arginine	1.000
Sodium pyruvate	1.000
Hemin	0.005
Menadione	0.0005
Agar	10.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Anaerobic bacteria are widespread in natural. They can be isolated from soil, marshes, lake and river sediments, oceans, sewage, food and animals. In humans & animals anaerobic bacteria under immunecompromised conditions are prevalent in the oral cavity around the teeth, in the gastrointestinal tract, in the orifices of the genitourinary tract and on the skin [1]. Also, anaerobic infections are often associated with tissue necrosis and abscess formation, failing of delivery of antimicrobial agents through blood to the actual site of infection. This explains why anaerobic infections are managed with debridement, aspiration and/or surgical removal of infected tissue. Because of the technical and interpretive difficulties associated with anaerobic susceptibility testing, formulation of definitive recommendations is difficult [2].
Wilkins Chalgren Anaerobic Agar Base, formulated by Wilkins and Chalgren [3], along with Brucella Agar Base is the preferred medium for agar dilution tests with anaerobes. This medium is also recommended for testing anaerobic bacteria [4-6]. Wilkins Chalgren Agar needs to be appropriately supplemented to support the growth of certain anaerobic bacteria. Hemin and Menadione (Vitamin K3) enhances the growth of *Bacteroides* species and *Prevotella melaninogenica ,* respectively and many other species of gram-negative anaerobic rods [8, 6]. The medium can also be supplemented with defibrinated or lysed blood for the growth of fastidious anaerobic bacteria [7].Peptic digest of animal tissues and casein enzymic hydrolysate serve as sources of essential nutrients including carbon andnitrogen. Yeast extract provides vitamins and other growth factors like purines and pyrimidines that are essential for the growth of P.melaninogenica . Arginine serves as an amino acid source while pyruvate serves as an energy source. The medium can be made selective for non-sporing anaerobic bacteria and gram-negative anaerobic bacteria by addition of Non-Spore Anaerobic Supplement (MS2001) and G. N. Spore Anaerobic Supplement (MS2002) respectively.

Methodology

Suspend 43.01 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Dispense and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C before adding antibiotics to be tested. Mix gently and pour into sterile Petri plates. For cultivation of anaerobes, aseptically add the rehydrated contents of 2 vials each of Non-Spore Anaerobic Supplement (MS2001) or G. N. Spore Anaerobic Supplement (MS2002) as desired to the sterile molten medium before pouring into sterile Petri plates.

Quality Control

Physical Appearance
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.0% Agar gel.
Colour and Clarity of prepared medium
Medium amber coloured clear to slightly opalescent gel forms in Petri plates.
Reaction
Reaction of 4.3% w/v aqueous solution at 25°C. pH : 7.1±0.2
pH range 6.90-7.30
Cultural Response/Characteristics
DM 1832: Cultural characteristics observed with added Non-spore Anaerobic Supplement (MS2001) or G.N.Spore Anaerobic Supplement(MS2002) under anaerobic condition, after an incubation at 35-37°C of 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Bacteroides fragilis ATCC 25285	50-100	luxuriant	>=50%
Clostridium perfringens ATCC 12924	50-100	Luxuriant	>=50%
Escherichia coli ATCC 25922	>=10^3	inhibited	0%
Prevotella melaninogenicus ATCC 15930	50-100	luxuriant	>=50%

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1.Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippinccott Company.
2.Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
3.Wilkins T. D. and Chalgren S., 1976, Antimicrob. Agents Chemother., 10 : 926.
4.King A., Phillips I., 1988, J. Antimicrob. Chemother., 21:425-438.
5.Clinical and Laboratory Standards Institute, 2006, Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria, Approved standard M11-A3, CLSI, Villanova, Pa.
6.Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.
7.Gibbons R. J. and MacDonald J. B., 1960, J. Bacteriol., 80:164.
8.Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.

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