



Wilkins Chalgren Anaerobic Broth Base

M863

Wilkins Chalgren Anaerobic Broth Base is used for cultivation and susceptibility testing of anaerobic bacteria.

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
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 33.0 grams in 1000 ml distilled water. Heat if necessary 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 dispense into sterile tubes. For cultivation of anaerobes, aseptically add the rehydrated contents of 2 vials each of Non-Spore Anaerobic Supplement (FD001) or G. N. Spore Anaerobic Supplement (FD002) as desired to the sterile molten medium before dispensing into sterile tubes.

Principle And Interpretation

Wilkins Chalgren Anaerobic Broth Base, formulated by Wilkins and Chalgren (1), is the preferred medium for susceptibility testing of anaerobes. This medium is also recommended for testing anaerobic bacteria (2, 3, 4). Wilkins Chalgren Anaerobic Broth Base is similar to the agar medium, except the agar (5). The broth medium is especially useful in the broth micro-dilution tests (6). Wilkins Chalgren Broth media need 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 (7, 4). The medium can also be supplemented with defibrinated or lysed blood for the growth of fastidious anaerobic bacteria (5).

Peptic digest of animal tissues and casein enzymic hydrolysate serve as sources of essential nutrients including carbon and nitrogen. 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 (FD001) and G. N. Spore Anaerobic Supplement (FD002) respectively.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Medium amber coloured clear solution in tubes.

Reaction

Reaction of 3.3% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

M863: Cultural characteristics observed with added Non-Spore Anaerobic Supplement (FD001) or G.N.Spore Anaerobic Supplement (FD002) Under anaerobic conditions, after an incubation at 35-37°C of 48 hours.

Organism	Inoculum (CFU)	Growth
<i>Bacteroides fragilis</i> ATCC 25285	50-100	luxuriant
<i>Clostridium perfringens</i> ATCC 12924	50-100	luxuriant
<i>Prevotella melaninogenicus</i> ATCC 15930	50-100	luxuriant
<i>Escherichia coli</i> ATCC 25922	$\geq 10^3$	inhibited

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Wilkins T. D. and Chalgren S., 1976, Antimicrob. Agents Chemother., 10 : 926
2. King A., Phillips I., 1988, J. Antimicrob. Chemother., 21:425-438
3. Clinical and Laboratory Standards Institute, 2006, Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria, Approved standard M11-A3, CLSI, Villanova, Pa.
4. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone
5. Gibbons R. J. and MacDonald J. B., 1960, J. Bacteriol., 80:164.
6. Quinto G. and Sebald M., 1964, Am. J. Med. Technol., 30:381.
7. Isenberg (Ed.), 2004, Clinical Microbiology Procedures Handbook, Vol. 3, American Society for Microbiology, Washington. D.C.

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