Technical Information

Charcoal Blood Agar Base

Product Code: DM 1646

Application: - Charcoal Blood Agar Base is recommended for the cultivation of *Bordetella pertussis* for vaccine production and also for the maintenance of stock cultures.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Beef extract	10.000
Starch, soluble	10.000
Sodium chloride	5.000
Charcoal	4.000
Yeast extract	3.500
Agar	12.000
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

The genus Bordetella contains has species : Bordetella pertussis, Bordetella parapertussis, Bordetella bronchiseptica and Bordetella avium[1]*. Genetic studies have shown that these organisms are very closely related to each other. Humans are the only host for B.pertussis and B.parapertussis, while B.bronchoseptica is found in a wide variety of animals and in humans*[2]*. B.avium is found in birds. Bordetella species are obligately aerobic and metabolically not very active. Except B.bronchoseptica all are none-motile .B.pertussis is the major cause of whooping cough or pertussis in human beings.*

B.parapertussis is associated with a milder form of the disease[3]*. Primary isolation of B.pertussis requires an enrichment media in which the addition of charcoal and 15-20% blood neutralize the growth-inhibiting effects of the media.*

Charcoal Agar is prepared as per method described by Mishulow, Sharpe and Cohen[2]*. This medium can be used as a replacement for Bordet-Gengou Agar for isolation of B.pertussis from clinical specimens and for the production of B. pertussis vaccines. Charcoal Agar supplemented with horse blood can also be used for the cultivation and isolation of Haemophilus influenzae*[4]

The difficulty in the isolation of Bordetella pertussis from nasopharyngeal secretions is the inhibition of normal microflora during the long incubation period on nutritious media. Penicillin is added to the medium as an antimicrobial agent for restricting the growth of other contaminants. However Penicillin resistant floras still cause contamination that was observed by Lacey[4]*. To overcome this problem he supplemented penicillin with diamidino-diphenylamine dihydrochloride, The selectivity of the medium was further increashed by adding methicilin by Broome et.al.* [5]*. Sutcliffe and Abbott found that Cephalexin was still better than Methicillin (6) in supressinyg unwanted nasopharynheal flora associated with clinical specimen.*

Regan and Lowe (7) have further showed that Charcoal Blood Agar Base with half strength of cephalexin is an excellent selective enrichment transport medium. Cephalexin is added to inhibit gram-positive organisms that may be present in specimen as cant aminant. Both non-selective and selective media should be inoculated since some stains of B. pertussis may be slightly inhibited by cephalexin. Charcoal Blood Agar Base is used for the cultivation of B.pertussis for vaccine production.

Medium ingredients like peptic digest of animal tissue, beef extract and yeast extract provide essential nutrients to the organisms. Sodium chloride maintains osmotic balance. Starch soluble and charcoal neutralizes substances toxic to Bordetella species such as fatty acids. As charcoal has the tendency to settle at the bottom of the flask swirl the flasks gently to obtain a uniform charcoal suspension while ponring media in petriplates/testtubes[8].

The medium can also be used for the maintenance of stock cultures of Bordetella pertussis on slants with weekly subcultures.

Methodology

Suspend 54.5 grams of powder media in 900 ml distilled water. Shake well & heat to boil to dissolve the medium with frequent stirring. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. For 100 ml of the medium add 10 ml of sterile defibrinated horse blood, 0.3 ml of sterile 100 u/ml Penicillin solution and 0.3 ml of 0.1% solution of 4:4 Diamido-diphenylamine

Quality Control

Physical Appearance
Grey to greyish black homogeneous free flowing powder

Gelling
Firm, comparable with 1.2% Agar gel

Colour and Clarity of prepared medium
Black coloured, opaque gel with undissolved black particles forms in Petri plates

Reaction
Reaction of 5.45% w/v aqueous solution at 25°C. pH : 7.5±0.2
pH range 7.30-7.70

Cultural Response/ characteristices
DM 1646: Cultural characteristics observed w/added sterile defibrinated blood and 100u/ml penicillin solution and 0.1% solution of 4:4 Diamido-diphenylamine hydrochloride, after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Bordetella bronchiseptica ATCC 4617	50-100	good-luxuriant	>=50%
Bordetella parapertussis ATCC 15311	50-100	good-luxuriant	>=50%
Bordetella pert ussis ATCC 8467	50-100	good-luxuriant	>=50%
Staphylococcus aureus ATCC 25923	50-100	inhibited	0%
Klebsiella pneumoniae ATCC 13883	50-100	inhibited	0%

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^{o} in sealable plastic bags for 2-5 days.

Further Reading

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and YolkenR. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
2. Mishulow, Sharpe and Cohen, 1953, Am. J. Public Health, 43:1466
3. Linneman and Pery, 1977, Am. J. Dis. Child., 131:560.
4. Lacey B. W., 1954, J. Hyg., 59:273.
5. Broome C. V., Fraser D. W. and English J. W., 1979, Internat. Symp. on Pertussis DHEW J., Washington D.C., pp 19-29.
6. Sutcliffe E. M. and Abbott J. D., 1979, B.M.J. II: 732-733.
7. Regan and Lowe F., 1977, J. Clin. Microbiol., 6:303.
8. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification -Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.

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