Technical Information

Charcoal Agar Base with Niacin

Product Code: DM 2053

Application: - Charcoal Agar Base with Niacin is recommended for the cultivation of *Bordetella pertussis* and *Haemophilus infuenzae*

Composition**

Ingredients	Gms / Litre
Pancreatic digest of gelatin	10.000
Beef extract	10.000
Sodium chloride	5.000
Starch	10.000
Nicotinic acid (Niacin)	0.001
Charcoal	4.000
Agar	12.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

The genus Bordetella has four species : Bordetella pertussis, Bordetella parapertussis, Bordetella bronchiseptica and Bordetella avium [1]. Genetic studies have shown that these organisms are related closely to each other. Humans are the only host for B.pertussis and B.parapertussis , while B.bronchoseptica is in a wide variety of animals and occasionally found in humans[2]. B. avium is found in birds. Bordetella species are obligately aerobic and metabolically not very active. All are non-motile except B.bronchoseptica .

B.pertussis is the major cause of whooping cough (pertussis.). B.parapertussis is associated with a milder form of the disease (3). Primary isolation of B.pertussis in particular, requires the addition of charcoal and 15-20% blood to neutralize the growth-inhibiting effects.

Charcoal Agar is prepared according to the method of Mishulow, Sharpe and Cohen[2]. This medium can be used as a replacement for Bordet-Gengou Agar for isolation of B.pertussis 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].

Medium ingredients like pancreatic digest of gelatin and beef 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. Charcoal has the tendency to settle at the bottom of the flask. Therefore, before dispensing, swirl the flasks gently to obtain a uniform charcoal suspension[7].

The difficulty in the isolation of Bordetella pertussis from nasopharyngeal secretions is the repression of unwanted flora during the long incubation period on nutritious media. Penicillin can be added to the medium as an antimicrobial agent for restricting the other contaminants. However Penicillin resistant flora still causes the contamination that was observed by Lacey[4]. Necessity of the Nicotinic acid as a growth factor was showed by Proom[8]. Methicillin was found to be superior to Penicillin in suppressing unwanted nasopharyngeal flora as observed by Broome et al[5]. Sutcliffe and Abbott[6] found that Cephalexin was still better than Methicillin.

The medium can also be used for the maintenance of stock cultures of *Bordetella pertussis* on slants with weekly subcultures. Charcoal Agar with Niacin can be converted to Chocolate Agar for isolation of *Ha em op hilus* species.

Methodology

Suspend 51.0 grams in 900 ml distilled water. Heat to boiling to dissolve the medium with frequent stirring. Sterilize by autoclaving at 15 lbs pressure (12 1°C) for 15 minutes. Cool to 50°C and aseptically add sterile 10 % of defibrinated blood and rehydrated contents of one vial of Bordetella Selective Supplement (MS2004). Mix well and pour into sterile Petri plates. For *Haemophilus* species the medium can be converted to chocolate agar.

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 5.1% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH range 7.20-7.60

Cultural Response/ characteristices

DM 2053: Cultural characteristics observed with added sterile defibrinated blood and Bordetella Selective Supplement (MS2004), 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	>=10^3	inhibited	0%
Klebsiella pneumoniae ATCC 13883	>=10^3	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. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification -Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
8. Proom H., 1955, J. Gen. Microbiol., 12 (1): 63-75.

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