PRODUCT SPECIFICATION SHEET

Charcoal Agar Base (DM059)

Intended Use
Charcoal Agar Base is used for cultivation in vaccine production and stock culture maintenance of *Bordetella pertussis*

Product Summary and Explanation
Charcoal Agar is prepared according to the method of Mishulow, Sharpe and Cohen.[1] The authors found this medium to be an efficient substitute for Bordet-Gengou Agar in the production of B. pertussis vaccines.
The genus *Bordetella* consists primarily of four species: *Bordetella pertussis*, *B. parapertussis*, *B. bronchiseptica* and *B. avium;* additional species have recently been described.[2] Genetic studies have shown that these organisms are very closely related to each other. All *Bordetella* are respiratory pathogens, residing on the mucous membranes of the respiratory tract. Humans are the only host of *B. pertussis* and *B.parapertussis* , while *B.bronchoseptica* is found in a wide variety of animals and occasionally found in humans[1] . *B.avium* is found in birds. *Bordetella* species are obligately aerobic and metabolically not very active. They are non-motile except *B. bronchoseptica* . *B.pertussis* is the major cause of whooping cough or 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, 15-20% blood to neutralize the growth-inhibiting effects. Isolation of this organism requires enrichment medium.

Principles of the Procedure
The nitrogen and carbon sources as well as the amino acids are provided by Beef Heart Infusion Solids and Enzymatic Digest of Gelatin. Yeast Extract is the vitamin source in this medium. Sodium Chloride maintains the osmotic environment. Soluble Starch and charcoal absorb toxic metabolites to neutralize substances toxic to *Bordetella species*, such as fatty acids. 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 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 floras still cause contamination, which as observed by Lacey[4]. Methicillin was found to be superior than Penicillin in suppressing unwanted nasopharyngeal flora as observed by Broome et al[5] . Sutcliffe and Abbott found that Cephalexin was still better than Methicillin[6]. are observed on plates. Confirm the findings with DFA i.e. Direct Fluorescent Antibody testing. To make earlier diagnosis, perform direct fluorescent antibody testing on the secretion.

Formula / Liter

Ingredients	Gms / Liter
Beef heart, infusion from	500.00
Peptic digest of animal tissue	10.00
Yeast extract	3.50
Starch, soluble	10.00
Charcoal	4.00
Sodium chloride	5.00
Agar	18.00
Final pH: 7.3 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 31.25 grams in 450 ml distilled water.
2.Heat to boiling to dissolve the medium with frequent stirring.

3.Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C.
4.Aseptically add sterile 10% of defibrinated blood and rehydrated contents of 1 vial of Bordetella Selective Supplement (MS003). Charcoal Agar can be converted to Chocolate Agar for isolation of *Haemophilus* species.

Quality Control Specifications

Dehydrated Appearance	Grey to greyish black colored, homogeneous, free flowing powder
Prepared Medium	Black coloured, opaque gel with undissolved black particles forms in Petri plates
Reaction of 6.25% Solution	pH : 7.3 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.8% Agar gel

Expected Cultural Response: Cultural characteristics observed with added sterile defibrinated blood and Bordetella Selective Supplement (MS003), after an incubation at 35 - 37°C for 24 - 48 hours

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Recovery
1.	*Bordetella bronchiseptica ATCC 4617*	50 -100	Good-luxuriant	>=50%
2.	*Bordetella parapertussis ATCC 15311*	50 -100	Good-luxuriant	>=50%
3.	*Bordetella pertussis ATCC 8467*	50-100	Good-luxuriant	>=50%
4.	*Staphylococcus aureus ATCC 25923*	>=10^3	Inhibited	0%
5.	*Klebsiella pneumoniae ATCC 13883*	>=10^3	Inhibited	0%

The organisms listed are the minimum that should be used for quality control testing.

Bordetella bronchiseptica ATCC 4617

Test Procedure
Refer to appropriate references for a complete discussion on isolation, identification, and maintenance of Bordetella spp. and other fastidious microorganisms.[7,8]

Results
Refer to appropriate references for results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Charcoal has a tendency to settle out of the medium. Swirl the flask gently when dispensing to obtain a uniform charcoal suspension.[9]

Packaging

Product Name : Charcoal Agar Base
Product Code : DM059
Available Pack sizes : 100gm / 500gm

References

1. Mishulow, Sharpe and Cohen. 1953. Am. J. Public Health, 43:1466.
2. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
3. Ensminger P. W., Gulberston C. G. and Powell H. M., 1953. J. Infect. Dis., 93(3):266.
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. Marcon, M. J. 1995. Bordetella, p. 566-573. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.).,Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
8. Isenberg, H. D. (ed.). 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
9. MacFaddin, J. D. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 110-114. Williams & Wilkins, Baltimore, MD.

Further Information

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