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Charcoal Blood Agar Base (DM1054)

Intended Use
Charcoal Blood Agar Base (DM1054) is recommended for cultivation in vaccine production and stock culture maintenance of *Bordetella pertusis*.

Product Summary and Explanation
All *Bordetella* are respiratory pathogens, residing on the mucous membranes of the respiratory tract. The genus *Bordetella* contains four species: *Bordetella pertussis, Bordetella parapertussis, Bordetella bronchiseptica* and *Bordetella avium;* additional species have recently been described.[1] *Bordetella* species are obligately aerobic and metabolically not very active. They are non-motile except *B.bronchoseptica*. Genetic studies have shown that these organisms are very closely related to each other. *B.pertussis* is found only in humans and is the major cause of whooping cough or pertussis. *B.parapertussis* is associated with a milder form of the disease. *B. bronchiseptica* is an opportunistic human pathogen associated with both respiratory and non-respiratory infections, often occurring in patients having close contact with animals.[2] *B.avium* is found in birds. *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.
Charcoal Agar is prepared according to the method of Mishulow, Sharpe and Cohen. The authors found this medium to be an efficient substitute for Bordet-Gengou Agar for isolation of *B.pertussis* and for the production of *B. pertussis* vaccines. This medium can be used as a replacement for Bordet-Gengou Agar for isolation of *B.pertussis* and for the production of *B. pertussis* vaccine. Charcoal Agar supplemented with horse blood can also be used for the cultivation and isolation of *Haemophilus influenzae*.[4]
The inhibition of associated flora during the long incubation period on nutritious media makes the isolation of *Bordetella pertussis* from nasopharyngeal secretions difficult. Penicillin is added to the medium as an antimicrobial agent for restricting the other contaminants. However, Lacey observed contamination by the Penicillin resistant floras.[4] He therefore supplemented penicillin with diamidino-diphenylamine dihydrochloride, thereby increasing the selectivity of the medium. Broome et.al.[5] discovered that Methicillin was superior to Penicillin in suppressing unwanted nasopharyngeal flora. Sutcliffe and Abbott found that Cephalexin was still better than Methicillin.[6]
Further, Regan and Lowe[7] have showed that Charcoal Blood Agar Base of half strength with cephalexin is an excellent selective enrichment transport medium. Addition of Cephalexin helps to inhibit contaminant gram-positive organisms that may be present in specimen. Both non-selective and selective media should be inoculated since some *B. pertussis* strains may be slightly inhibited by cephalexin. Charcoal Blood Agar Base is used for the cultivation of *B.pertussis* for vaccine production. The medium can also be used for the maintenance of stock cultures of *Bordetella pertussis* on slants with weekly subcultures.

Principles of the Procedure
Charcoal Blood Agar Base contains peptic digest of animal tissue, beef extract and yeast extract which provide the nitrogen, carbon and amino acids essential for growth of the organisms. Yeast extract is a vitamin source. Sodium chloride helps to maintain the osmotic balance. Starch soluble and charcoal neutralizes substances toxic to *Bordetella* species such as fatty acids.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	10.00
Beef extract	10.00
Starch, soluble	10.00
Sodium chloride	5.00
Charcoal	4.00
Yeast extract	3.50

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Agar	12.00
Final pH: 7.5 ± 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 54.5 grams of the medium in one litre of distilled water.
2. Heat to boiling to dissolve the medium with frequent stirring.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 45-50°C. 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 hydrochloride per 100 ml of the medium.

Quality Control Specifications

Dehydrated Appearance	Grey to greyish black homogeneous free flowing powder
Prepared Medium	Black coloured, opaque gel with undissolved black particles forms in Petri plates
Reaction of 5.45% solution	pH : 7.5 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.2% Agar gel

Expected Cultural Response: 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.

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.

Test Procedure

Refer appropriate references for specific test procedures.

Results

Refer appropriate references and test procedures for interpretation of 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.

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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. Charcoal has a tendency to settle out of the medium. Swirl the flask gently when dispensing to obtain a uniform charcoal suspension.[8]
2. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
3. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Charcoal Blood Agar Base
Product Code : DM1054
Available Pack sizes : 500gm

References

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Further Information

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