Charcoal Agar Base

M344

Charcoal 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
Beef heart, infusion from	500.000
Peptic digest of animal tissue	10.000
Yeast extract	3.500
Starch, soluble	10.000
Charcoal	4.000
Sodium chloride	5.000
Agar	18.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.25 grams in 450 ml distilled water. Heat to boiling to dissolve the medium with frequent stirring. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add sterile 10% of defibrinated blood and rehydrated contents of 1 vial of Bordetella Selective Supplement (FD004). Mix well and pour into sterile Petri plates.Charcoal Agar can be converted to Chocolate Agar for isolation of *Haemophilus* species.

Principle And Interpretation

The genus *Bordetella* contains four 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 of *B. pertussis* and *B.parapertussis* , while *B.bronchoseptica* is found 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. 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.

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).

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).

The ingredients like beef heart infusion, peptic digest of animal tissue, 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. 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).

Technique (8): Collect the nasal swabs in early stage of the illness and place in tubes of half strength Charcoal Agar Base supplemented with 10% v/v lysed defibrinated horse blood and Bordetella Selective Supplement (FD004). Generously inoculate the swabs on to thick layer of Charcoal Agar Base containing 10% v/v blood and Bordetella Selective Supplement (FD004). Non-selective medium (without FD004) may be used in addition. Replace the swab in the original transport medium and hold at room temperature. Incubate the plates at 35°C in a moist atmosphere (60-70% humidity) upto 6 days. Examine plates after 40 hours incubation and twice daily thereafter. Small shiny grayish white, round corner, colonies of *Bordetella* species 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.

Quality Control

Appearance
Grey to greyish black homogeneous free flowing powder

Gelling
Firm, comparable with 1.8% Agar gel

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

Reaction
Reaction of 6.25% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
M344: Cultural characteristics observed with added sterile defibrinated blood and Bordetella Selective Supplement (FD004), after an incubation at 35 - 37°C for 24 - 48 hours

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
Bordetella bronchiseptica ATCC 4617	50-100	good-luxuriant	>=50%
Bordetella parapertussis ATCC 15311	50-100	good-luxuriant	>=50%
Bordetella pertussis 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

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

Reference

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