

Charcoal agar base

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

REF: BS.1/BBT01.100.0100	100 Gram	REF: BS.1/BBT01.250.0250	250 Gram
REF: BS.1/BBT01.500.0500	500 Gram		

CLINICAL SIGNIFICANCE

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. bronchiseptica* 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. bronchiseptica*. *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.

METHOD PRINCIPLE

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 nutritive 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 to Penicillin in suppressing unwanted nasopharyngeal flora as observed by Broome et al (5). Sutcliffe and Abbott found that Cephalixin 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. Generously inoculate the swabs on to thick layer of Charcoal Agar Base containing 10% v/v blood and *Bordetella* Selective Supplement. Non-selective medium 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) up to 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.

MEDIA COMPOSITION

Item	Formula per liter of medium
- Beef heart, infusion from	500.0 gm
- Peptic digest of animal tissue	10.00 gm
- Yeast extract	3.500 gm
- Starch, soluble	10.00 gm
- Charcoal	4.000 gm
- Sodium chloride	5.000 gm
- Agar	18.00 gm

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
 - S56:** dispose of this material and its container at hazardous or special waste collection point.
 - S57:** use appropriate container to avoid environmental contamination.
 - S61:** avoid release in environment.

For further information, refer to the Charcoal agar base material safety data sheet.

STORAGE AND STABILITY

BioScien Charcoal agar base are stable until expiration date stated on label when properly stored Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Final pH 7.3 ± 0.2 at 25°C

MEDIA PREPARATION

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. Mix well and pour into sterile Petri plates. Charcoal Agar can be converted to Chocolate Agar for isolation of *Haemophilus* species.

