



## Charcoal Blood Agar Base

M646

Charcoal Blood 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
Peptic digest of animal tissue	10.000
Beef extract	10.000
Starch, soluble	10.000
Sodium chloride	5.000
Charcoal	4.000
Yeast extract	3.500
Agar	12.000
Final pH ( at 25°C)	7.5±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 54.5 grams in 900 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. 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.

### 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 inhibition of associated flora during the long incubation period on nutritious media. Penicillin is added to the medium as an antimicrobial agent for restricting the other contaminants. However Penicillin resistant floras still cause contamination that was observed by Lacey (4). He therefore supplemented penicillin with diamidino-diphenylamine dihydrochloride, thereby increasing the selectivity of the medium. 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 Cephalexin was still better than Methicillin (6).

Regan and Lowe (7) have further showed that Charcoal Blood Agar Base of half strength with cephalexin is an excellent selective enrichment transport medium. Cephalexin is added 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.

Medium ingredients like peptic digest of animal tissue, beef extract and 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 (8).

The medium can also be used for the maintenance of stock cultures of *Bordetella pertussis* on slants with weekly subcultures.

## Quality Control

### 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 5.45% w/v aqueous solution at 25°C. pH : 7.5±0.2

### pH

7.30-7.70

### Cultural Response

M646: 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.

Organism	Inoculum (CFU)	Growth	Recovery
<i>Bordetella bronchiseptica</i> ATCC 4617	50-100	good-luxuriant	≥50%
<i>Bordetella parapertussis</i> ATCC 15311	50-100	good-luxuriant	≥50%
<i>Bordetella pertussis</i> ATCC 8467	50-100	good-luxuriant	≥50%
<i>Staphylococcus aureus</i> ATCC 25923	≥10 <sup>3</sup>	inhibited	0%
<i>Klebsiella pneumoniae</i> ATCC 13883	≥10 <sup>3</sup>	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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- Mishulow, Sharpe and Cohen, 1953, Am. J. Public Health, 43:1466
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