# Technical Information

## Charcoal Agar Base

## Product Code: DM 1344

**Application: -** 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

## Principle & Interpretation

The genus Bordetella comprised of 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 for B. pertussis and B.parapertussis, while B.bronchoseptica is found in a large variety of animals and rarely 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 and 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.

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].

For isolation of B.perthssis from suspected cow whooping cough 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 (MS2004). Generously inoculate the swabs on to thick layer of Charcoal Agar Base containing 10% v/v blood and Bordetella Selective Supplement (MS2004). Non-selective medium (without MS2004) 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 [8].

## Methodology

Suspend 31.25 grams of powder media in 450 ml distilled water. Shake well & heat 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 (MS2004). Charcoal Agar can be converted to Chocolate Agar for isolation of *Haemophilus* species.

## Quality Control

**Physical 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 range** 7.10-7.50

**Cultural Response/ characteristices**
DM 1344: Cultural characteristics observed with added sterile defibrinated blood and Bordetella Selective Supplement ( MS2004),

| Organism | Inoculum (CFU) | Growth | Recovery |
|---|---|---|---|
| *Escherichia coli ATCC 25922* | 50-100 | good-luxuriant | >=50% |
| *Enterococcus faecalis A TCC 29212* | 50-100 | good-luxuriant | >=50% |
| *Klebsiella pneumoniae ATCC 13883* | 50-100 | good-luxuriant | >=50% |
| *Proteus vulgaris ATCC 13315* | 50-100 | inhibited | 0% |
| *Staphylococcus aureus ATCC 25923* | 50-100 | inhibited | 0% |

## Storage and Shelf Life

**Dried Media :** Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
**Prepared Media** : 2-8° in sealable plastic bags for 2-5 days.

## Further Reading

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
2. Mishulow, Sharpe and Cohen, 1953, Am. J. Public Health, 43:1466.
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. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
8. Young S. A., Anderson G. L. and Mitchell P. D., 1987, Clin. Microbiol. Newsletter, 9, 176-179.

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