

TM 062 – CHARCOAL AGAR BASE

INTENDED USE

For cultivation of fastidious microorganisms like *Bordetella pertussis* for vaccine production.

PRODUCT SUMMARY AND EXPLANATION

The genus *Bordetella* contains four species: *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica* and *Bordetella avium*. 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. *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. 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. 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*.

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. Methicillin was found to be superior than Penicillin in suppressing unwanted nasopharyngeal flora as observed by Broome et al. Sutcliffe and Abbott found that Cephalexin was still better than Methicillin.

Examine plates after 40 hours of 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.

COMPOSITION

Ingredients	Gms / Ltr
Beef heart infusion from	500.000
Peptone	10.000
Yeast extract	3.500
Starch, soluble	10.000
Charcoal	4.000
Sodium chloride	5.000
Agar	18.000

PRINCIPLE

The ingredients like beef infusion from, Peptone, 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.

INSTRUCTION FOR USE

- Dissolve 31.25 grams in 450 ml purified / distilled water.
- Heat to boiling to dissolve the medium with frequent stirring.
- Sterilize by autoclaving at 15 psi 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.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Grey to greyish black homogeneous free flowing powder.
Appearance of prepared medium	: Black coloured, opaque gel with undissolved black particles forms in Petri plates.
pH (at 25°C)	: 7.3±0.2

INTERPRETATION

Cultural characteristics observed after incubation d with added sterile defibrinated blood and Bordetella Selective Supplement.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Incubation Temperature	Incubation Period
<i>Bordetella bronchiseptica</i>	4617	50-100	Good-luxuriant	≥50%	35-37°C	24-48 Hours
<i>Bordetella parapertussis</i>	15311	50-100	Good-luxuriant	≥50%	35-37°C	24-48 Hours
<i>Bordetella pertussis</i>	8467	50-100	Good-luxuriant	≥50%	35-37°C	24-48 Hours
<i>Staphylococcus aureus subsp. aureus</i>	25923	≥10 ³	Inhibited	0%	35-37°C	24-48 Hours
<i>Klebsiella pneumoniae</i>	13883	≥10 ³	Inhibited	0%	35-37°C	24-48 Hours

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.













DISPOSAL



After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

1. Broome C. V., Fraser D. W. and English J. W., 1979, Internat. Symp. on Pertussis DHEW J., Washington D.C., pp 19-29.
2. Lacey B. W., 1954, J. Hyg., 59:273.
3. Linneman and Pery, 1977, Am. J. Dis. Child., 131:560.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification -Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
5. Mishulow, Sharpe and Cohen, 1953, Am. J. Public Health, 43:1466.
6. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Tenover F. C., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
7. Regan and Lowe F., 1977, J. Clin. Microbiol., 6:303.
8. Sutcliffe E. M. and Abbott J. D., 1979, B.M.J. II: 732-733.

 GMP Good Manufacturing Practices Certified	 IVD For In Vitro Diagnostic Use	 QTY. Quantity	 LOT/ B. NO. Lot / Batch Number	 REF Catalogue Number	 Manufacturer
 Temperature Unit	 EC REP Authorized Representative <small>MedNet GmbH Borkstrasse 10, 48163 Muenster, Germany</small>	 European Conformity	 QR Code	 Consults Instructions for Use	 Best Before

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**
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