# CM 20406 – 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.bronchoseptica 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. bronchoseptica. 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

Theingredients 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 FORUSE

Dissolve 31.25 gramsin 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

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

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

## PACKAGING:

Inpacksizeof500 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

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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 YolkenR. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
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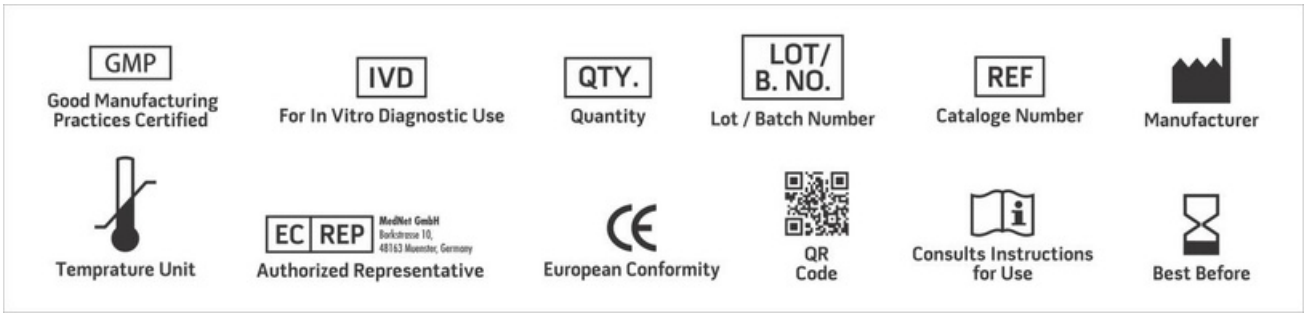


NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

*For LabUse Only