TM 1160 – CHARCOAL BLOOD AGAR BASE

INTENDED USE

For cultivation of *Bordetella pertussis* for vaccine production & also for maintenance of stock cultures.

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 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. 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.. Sutcliffe and Abbott found that Cephalexin was still better than Methicillin

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

COMPOSITION

Ingredients	Gms / Ltr
Peptone	10.000
Beef extract	10.000
Starch, soluble	10.000
Sodium chloride	5.000
Charcoal	4.000
Yeast extract	3.500
Agar	12.000

PRINCIPLE

Medium ingredients like peptone, 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.

INSTRUCTION FOR USE

- Dissolve 54.5 grams in 900 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. 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.

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) : 7.5±0.2

INTERPRETATION

Cultural characteristics observed after incubation w/added sterile defibrinated blood and 100u/ml penicillin solution and 0.1% solution of 4:4 Diamido-diphenylamine hydrochloride.

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:

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.

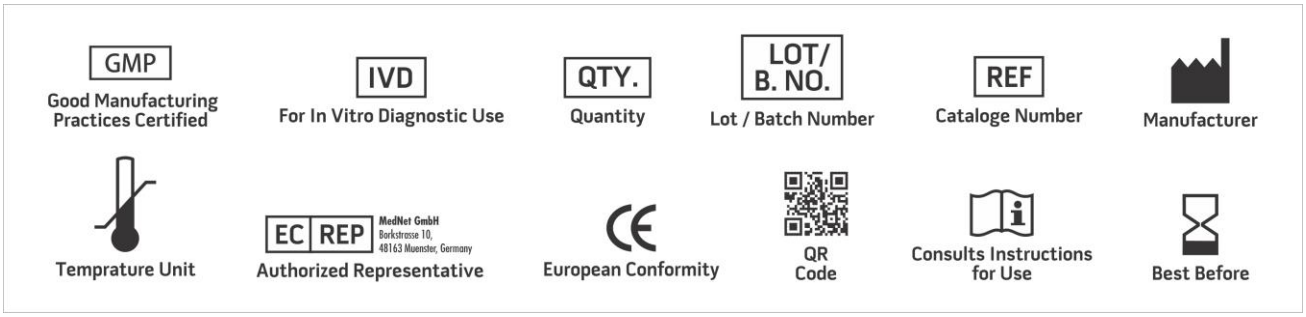
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

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 YolkenR. H., (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.



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

***For Lab Use Only**

Revision: 08 Nov., 2019