Charcoal Blood Agar Base

M646

Intended Use:

Recommended for the cultivation of *Bordetella pertussis* for vaccine production and also for the maintenance of stock cultures.

Composition**

Ingredients	g / L
Peptone	10.000
HM peptone B#	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
- Equivalent to Beef extract

Directions

Suspend 54.5 grams in 900 ml purified/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. Mix well and pour into sterile Petri plates.

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 peptone, HM peptone B 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.

Type of specimen

Clinical samples- nasopharyngeal swabs, throat swab, whooping cough, etc. Pure isolate for vaccine production

Specimen Collection and Handling:

For clinical samples, follow appropriate techniques for handling specimens as per established guidelines (9,10).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Swirl the flask gently when dispensing to obtain a uniform charcoal suspension.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within expiry period the when stored at recommended temperature.

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

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
Bordetella bronchiseptica ATCC 4617	50-100	good-luxuriant	>=50%
Bordetella parapertussis ATCC 15311	50-100	good-luxuriant	>=50%
Bordetella pertussis ATCC 8467	50-100	good-luxuriant	>=50%
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%
Klebsiella pneumoniae ATCC 13883 (00097*)	>=10^4	inhibited	0%

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

Reference

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9. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
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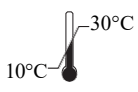
IVD ***In vitro*** **diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
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CE **CE Marking**

Do not use if package is damaged

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