Charcoal Agar Base

M344

Intended Use:

Recommended for cultivation of *Bordetella pertussis*, for vaccine production and also for stock culture maintenance.

Composition**

Ingredients	g / L
HM infusion B from 500g #	12.000
Peptone	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

#Equivalent to beef heart, infusion from

Directions

Suspend 31.25 grams in 450 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50° and aseptically add sterile 10% of defibrinated blood and rehydrated contents of one vial of Bos Selective Supplement (FD004).Mix well and pour into sterile Petri plates. For *Haemophilus* species, the medium can be converted to Chocolate Agar.

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 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 HM infusion B 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 (7).

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.

Type of specimen

Nasal swabs

Specimen Collection and Handling:

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 Bos Selective Supplement (FD004). Generously inoculate the swabs on to thick layer of Charcoal Agar Base containing 10% v/v blood and Bos Selective Supplement (FD004). Non-selective medium (without FD004) 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. 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. Confirm the findings with DFA i.e. Direct Fluorescent Antibody testing.

Performance and Evaluation

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

Quality Control

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

7.10-7.50

Cultural Response

Cultural characteristics observed with added sterile defibrinated blood and Bos Selective Supplement (FD004), 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 (8,9).

Reference

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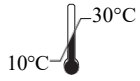
IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE Marking

Do not use if package is damaged

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