Bg`qbn`k @f`q A`rd v. Mh`bhm

M1053

Hmsdmcdc Trd9

Recommended for cultivation and isolation of *Bordetella pertussis* and *Haemophilus influenzae.*

Composition**

Ingredients	Gms / Litre
Gelatin peptone	10.000
HM peptone B #	10.000
Sodium chloride	5.000
Starch, soluble	10.000
Nicotinic acid (Niacin)	0.001
Charcoal	4.000
Agar	12.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 51.0 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 and aseptically add sterile 10 % of defibrinated blood and rehydrated contents of one vial of Bordetella 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).

Medium ingredients like gelatin peptone and HM peptone B provide nitrogen and carbon compounds, long chain amino acids and other 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).

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 flora still causes the contamination that was observed by Lacey (4). Necessity of the Nicotinic acid as a growth factor was showed by Proom (8). 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).

The medium can also be used for the maintenance of stock cultures of *Bordetella pertussis* on slants with weekly subcultures. Charcoal Agar with Niacin can be converted to Chocolate Agar for isolation of *Haemophilus* species.

Type of specimen

Clinical samples : sputum.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic 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. Some *Haemophilus* species will grow on *Bordetella* isolation media and cross-react with *B. pertussis* antisera.
2. *B. pertussis* colonies may not be visible without the aid of a microscope for 2-4 days.

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 5.1% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed with added sterile defibrinated blood and Bordetella Selective Supplement (FD004), after an incubation at 35 - 37°C for 24 - 48 hours

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
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 ATCC 25923	>=10^4	inhibited	0%
Klebsiella pneumoniae ATCC 13883	>=10^4	inhibited	0%

Reference

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