

Technical Information

Bordet Gengou Agar base w/1.6% Agar

Product Code: DM 1175A

Application: Bordet Gengou Agar base w/1.6% Agar is recommended for the detection and isolation of *Bordetella pertussis* and *Bordetella parapertussis*. Also used for the "cough plate" method in case of whooping cough.

Composition**

Ingredients	Gms / Litre
Potatoes, infusion from	125.000
Peptic digest of animal tissue	10.000
Sodium chloride	5.500
Agar	16.000
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Bordet Gengou Agar Media were initially devised by Bordet and Gengou⁽¹⁾ for cultivation of *Bordetella* species. Bordet Gengou Agar Base w/1.6% Agar is similar in composition to Bordet Gengou Agar Base with the exception being the difference in agar concentration.

Bordetella pertussis is the causative agent of whooping cough which can be isolated from pharyngeal extracts, nasopharyngeal secretions and pre-nasal swabs with the help of cough-plate technique. Kendrick and Eldering⁽²⁾ modified the original media by replacing 50% human or rabbit blood with 15% sheep blood to make the medium more enriched for detection of *B. pertussis* by the virtue of its haemolytic reaction. Enrichment of the basal media with 25% human blood helps in the detection of *Mycobacterium* species from small sputum inocula and in Streptomycin sensitivity testing⁽³⁾.

The medium is highly nutritious thus supports luxuriant growth of *Bordetella* species and can also be used for mass cultivation of *B. pertussis* for vaccine production⁽²⁾ and for maintaining stock cultures⁽¹⁾.

Potato infusion and peptic digest of animal tissue serve as carbon and nitrogen source while glycerol and blood enrichment provides additional nutrients. Sodium chloride maintains osmotic equilibrium. Incubation should be carried out in a moist chamber (60% humidity) at 37°C for upto 7 days. Medium should not be over dried before use. After 40 hours *B. pertussis* colonies appear smooth, raised, glistening with a zone of haemolysis. Some strains of *Bordetella* are not haemolytic. For confirmation, serodiagnosis and biochemical test should be performed. This medium can be made more selective for *Bordetella*, by using antibiotics like penicillin⁽⁴⁾, methicillin⁽⁵⁾, cephalexin⁽⁶⁾ of which, cephalexin was found to be superior. Cephalexin suppresses unwanted nasopharyngeal growth and significantly increases the isolation rate of *Bordetella* species. Cephalexin is used at a concentration of 40 mg/litre (MS2004). Amphotericin B (10 µg/ml) can be added as an antifungal agent to the medium.

For isolation of *B. pertussis* from specimens, use standard procedures. Incubate the plates in a moist chamber at 35-37°C for 7 days and examine daily with or without dissecting microscope (oblique illumination) to detect the presence of *B. pertussis*. Sometimes the accompanying mold colonies can mask the *B. pertussis* colonies. Use sterile scalpel or needle to remove the portion of the agar that contains spreading colonies of moulds. *B. pertussis* colonies may not be visible without microscope for 2-4 days. After 7 days of incubation plates may be discarded as negative. Some *Haemophilus* species will grow on *Bordetella* isolation media and cross-react with *B. pertussis* antisera. It may be prudent to rule out X and V factor dependence.

Methodology

Suspend 36 grams of powder media in 1000 ml distilled water containing 10 ml glycerol. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 15-20% sterile, fresh defibrinated blood (sheep, rabbit, human or human or horse). For selectivity aseptically add rehydrated content of 2 vials of Bordetella Selective Supplement (MS2004). Mix thoroughly, taking care to avoid incorporation of air bubbles and pour into sterile Petri plates.

Quality Control

Physical Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.6% Agar gel.

Colour and Clarity of prepared medium

Basal medium: Light yellow coloured clear to slightly opalescent gel. After addition of 15-20% sterile defibrinated blood: Cherry red coloured opaque gel forms in Petri plates.

Reaction

Reaction of 3.6% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH range

6.50-6.90

Cultural Response/Characteristics

DM 1175A: Cultural characteristics observed with added 15-20% sterile defibrinated fresh blood and Bordetella Selective Supplement (MS2004), after an incubation at 35-37°C for 3-4 days.

Organism	Growth	Haemolysis
<i>Bordetella bronchiseptica</i> ATCC 4617	good-luxuriant	Gamma
<i>Bordetella parapertussis</i> ATCC 15311	good-luxuriant	gamma
<i>Bordetella pertussis</i> ATCC 8467	good-luxuriant	beta
<i>Staphylococcus aureus</i> ATCC 25923	inhibited	

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. Bordet and Gengou, 1906, Ann. Inst. Pasteur, 20:731.
2. Kendrick and Eldering, 1934, Am. J. Public Health, 24:309
3. Tarshis M. S. and Frisch A. W., 1951, Am. J. Clin. Pathol., 21:101.
4. Flemming A., 1932, J. Path. Bacteriol., 35:83 1.
5. Broome C. V., Fraser D. W. and English J. W., 1979, Internat. Symp. on Pertussis, DHEW, Washington, D.C., 19.
6. Suitcliffe E. M. and Abbott J. D., 1972, B. M. J., iii:732.

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