Bordet Gengou HiVeg™ Agar Base

MV175

Intended use

Recommended for the detection and isolation of *Bordetella pertussis* and *Bordetella parapertussis.*

Composition**

Ingredients	g / L
Potatoes, infusion from	125.000
HiVeg™ peptone	10.000
Sodium chloride	5.500
Agar	20.000
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.00 grams in 1000 ml purified / distilled water containing 10 ml glycerol. Heat to boiling 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 horse). For selectivity aseptically add rehydrated contents of 2 vials of BBos Selective Supplement (FD004). Mix thoroughly, taking care to avoid incorporation of air bubbles and pour into sterile Petri plates.

Principle And Interpretation

Bordet Gengou Agar Media were originally formulated by Bordet and Gengou (1) for cultivation of *Bordetella* species. *Bordetella pertussis* is the causative agent of whooping cough and with the help of cough-plate technique, *B. pertussis* can be isolated from pharyngeal extracts, nasopharyngeal secretions and pre-nasal swabs. 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 aids 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 (4) and for maintaining stock cultures (1). Bordet Gengou HiVeg™ Agar Baseis prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks. Potato infusion and HiVeg™ peptone serve as carbon and nitrogen source, amino acids 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 (5), methicillin (4), cephalexin (2) 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/liter (FD004). 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 the aid of a 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.

Type of specimen

Clinical samples -Whooping cough, pharyngeal extracts, nasopharyngeal secretions and pre-nasal swabs.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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 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.

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

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

Basal Medium : Light yellow coloured clear to slightly opalescent gel. After addition of glycerol and 15% v/v sterile defibrinated blood: Cherry red coloured opaque gel forms in Petri plates.

Reaction

Reaction of 4% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH

6.50-6.90

Cultural Response

Cultural characteristics observed with added Glycerol and 15% v/v sterile defibrinated blood and Bos Selective Supplement (FD004), after an incubation at 35-37°C for 3-4 days.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Bordetella bronchiseptica ATCC 4617	50-100	good-luxuriant	>=50%	gamma
Bordetella parapertussis ATCC 15311	50-100	good-luxuriant	>=50%	gamma
Bordetella pertussis ATCC 8467	50-100	good-luxuriant	>=50%	beta
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

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. Broome C. V., Fraser D. W. and English J. W., 1979, Internat. Symp. on Pertussis, DHEW, Washington, D.C., 19.

5. Flemming A., 1932, J. Path. Bacteriol., 35:831.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W.(2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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