Bordet Gengou Agar Plate w/25% Sheep blood

MP175SB

Intended use

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	g / L
Potatoes, infusion from	125.000
Peptone	10.000
Sodium chloride	5.500
Agar	20.000
Glycerol	10.000ml
Sheep blood	250.000 ml
Bos Selective Supplement (FD004) - 2 vials	
Cephalexin	40.000 mg
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

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).

Potato infusion and 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.

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 - 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 (5,6). Wear procedure mask when collecting the samples as *B.pertussis/parapertussis* is highly infectious. Nasopharyngeal (NP) swab or aspirate from all persons with suspected cases is collected. The swab is then transported top the labporatory and processed,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 pack. 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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Some *Haemophilus* species will grow on *Bordetella* isolation media and cross-react with *B. pertussis* antisera.
4. *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

Sterile Bordet Gengou Agar Plate w/25% Sheep blood in 90 mm disposable plates with smooth surface and absence of black particles/cracks/bubbles.

Colour of medium

Cherry red coloured opaque medium

Quantity of medium

25 ml of medium in 90 mm disposable plates.

pH

6.50-6.90

Sterility check

Passes release criteria

Cultural Response

Cultural characteristics observed 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*)	>=10^3	inhibited	0%	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 2-8°C. Use before expiry date on the label. 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 (5,6).

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

2°C 8°C **Storage temperature**

EC REP

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CE Marking

Do not use if package is damaged