

TM 1147 – BORDET GENGOU AGAR BASE W/ 1.6% AGAR

INTENDED USE

For detection and isolation of *Bordetella pertussis* and *Bordetella parapertussis*.

PRODUCT SUMMARY AND EXPLANATION

Bordet Gengou Agar Media were originally formulated by Bordet and Gengou 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 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 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. 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 and for maintaining stock cultures. 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, methicillin, cephalixin of which, cephalixin was found to be superior. Cephalixin suppresses unwanted nasopharyngeal growth and significantly increases the isolation rate of *Bordetella* species. Cephalixin is used at a concentration of 40 mg/liter. 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.

COMPOSITION

Ingredients	Gms / Ltr
Potatoes, infusion from	125.000
Peptone	10.000
Sodium chloride	5.500
Agar	16.000

PRINCIPLE

Potato infusion and peptone serve as carbon and nitrogen, long chain amino acids, source while glycerol and blood enrichment provides additional nutrients. Sodium chloride maintains osmotic equilibrium.

INSTRUCTION FOR USE

- Dissolve 36.0 grams in 1000 ml purified/distilled water containing 10 ml glycerol.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi 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 two vials of Bordetella Selective Supplement.



- Mix thoroughly, taking care to avoid incorporation of air bubbles and pour into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Cream to yellow Appearance homogeneous free flowing powder.
Appearance 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.
pH (at 25°C)	: 6.7±0.2

INTERPRETATION

Cultural characteristics observed after incubation with added 15-20% sterile defibrinated fresh blood and Bordetella Selective Supplement.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Haemolysis	Incubation Temperature	Incubation Period
<i>Bordetella bronchiseptica</i>	4617	50-100	Good-luxuriant	≥50%	Gamma	35-37°C	3-4 Days
<i>Bordetella parapertussis</i>	15311	50-100	Good-luxuriant	≥50%	Gamma	35-37°C	3-4 Days
<i>Bordetella pertussis</i>	8467	50-100	Good-luxuriant	≥50%	Beta	35-37°C	3-4 Days
<i>Staphylococcus aureus subsp.aureus</i>	25923	≥10 ⁴	Inhibited	0%	-	35-37°C	3-4 Days

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.













DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

- Bordet and Gengou, 1906, Ann. Inst. Pasteur, 20:731
- Broome C. V., Fraser D. W. and English J. W., 1979, Internat. Symp. on Pertussis, DHEW, Washington, D.C., 19.
- Flemming A., 1932, J. Path. Bacteriol., 35:831.
- Kendrick and Eldering, 1934, Am. J. Public Health, 24:309
- Suitcliffe E. M. and Abbott J. D., 1972, B. M. J., iii:732.
- Tarshis M. S. and Frisch A. W., 1951, Am. J. Clin. Pathol., 21:101.



 GMP Good Manufacturing Practices Certified	 IVD For In Vitro Diagnostic Use	 QTY. Quantity	 LOT/ B. NO. Lot / Batch Number	 REF Catalogue Number	 Manufacturer
 Temperature Unit	 EC REP Authorized Representative <small>MedNet GmbH Barkstrasse 10, 48143 Muenster, Germany</small>	 European Conformity	 QR Code	 Consults Instructions for Use	 Best Before

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**
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