PRODUCT SPECIFICATION SHEET

Bordet Gengou Agar Base (DM042)

Intended Use
Bordet Gengou Agar Base (DM042) is recommended for detection and isolation of *Bordetella pertussis* and *Bordetella parapertussis*.

Product Summary and Explanation
Bordetella pertussis, the etiologic agent of this disease, may be isolated from aspirated bronchial or nasopharyngeal secretions, perinasal swabs or, perhaps with greater difficulty due to the diversity of flora, from throat swabs.[1] *pertussis* is the causative agent of whooping cough and with the help of cough-plate technique. Bordet Gengou Agar Media were originally formulated by Bordet and Gengou[2] for cultivation of *Bordetella* species. In 1934, Kendrick and Eldering[3] 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.[4] 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[3] and for maintaining stock cultures.[2]

Principles of the Procedure
Bordet Gengou Agar Base contains potato infusion and peptic digest of animal tissue serve as carbon and nitrogen source while glycerol and blood enrichment provides additional nutrients to support the growth of *B. pertussis*. Sodium chloride maintains osmotic equilibrium. Defibrinated animal blood supplies additional nutrients and enables the detection of hemolytic reactions, which aid in the identification of *B. pertussis*. This medium can be made more selective for *Bordetella* , by using antibiotics like penicillin, methicillin, cephalexin 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 (MS003). Amphotericin B (10 µg/ml) can be added as an antifungal agent to the medium.

Formula / Liter

Ingredients	Gms / Liter
Potatoes, infusion from	125.00
Peptic digest of animal tissue	10.00
Sodium chloride	5.50
Agar	20.00
Final pH: 6.7 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 40 grams of the medium in one liter of distilled water containing 10 ml glycerol.
2. Heat if necessary, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 45-50°C and aseptically add 15 - 20% sterile, fresh defibrinated blood (sheep, rabbit, human or horse).
5. For selectivity aseptically add rehydrated contents of 2 vials of Bordetella Selective supplement (MS003).
6. Mix thoroughly, taking care to avoid incorporation of air bubbles and pour into sterile Petri plates.

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Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
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 of 4.00% Solution	pH : 6.7 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 2.0% Agar gel

Expected Cultural Response: Cultural characteristics observed with added Glycerol and 15% v/v sterile defibrinated blood and Bordetella Selective Supplement (MS003), after an incubation at 35-37°C for 3-4 days.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Haemolysis
1.	*Bordetella bronchiseptica ATCC 4617*	50 -100	good-luxuriant	>=50 %	Gamma
2.	*Bordetella parapertussis ATCC 15311*	50 -100	good-luxuriant	>=50 %	Gamma
3.	*Bordetella pertussis ATCC 8467*	50 -100	good-luxuriant	>=50 %	Beta
4.	*Staphylococcus aureus ATCC 25923*	>=10^3	Inhibited	0%	

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. For isolation of *B. pertussis* from specimens, use standard procedures.
2. 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*.
3. 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.
4. *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.

Results

1. *Bordetella pertussis* produces small, smmoth, raised, glistening colonies that resemble bisected pearls.
2. The colonies are usually surrounded by a zone of hemolysis; however, some strains of *B. pertussis* are not hemolytic.
3. Gram stains, biochemical tests and serological procedures should be performed to confirm findings.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Some *Haemophilus* spp. 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.
2. Consult appropriate texts for detailed information and recommended procedures.

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Packaging
Product Name : Bordet Gengou Agar Base
Product Code : DM042
Available Pack sizes : 100gm / 500gm

References

1. Loeffelholz and Sanden. 2007. *In* Murray, Baron, Jorgensen, Landry and Pfaller (ed.), Manual of clinical microbiology 9th ed. American Society for Microbiology, Washington, D.C.
2. Bordet and Gengou, 1906, Ann. Inst. Pasteur, 20:731.
3. Kendrick and Eldering, 1934, Am. J. Public Health, 24:309
4. Tarshis M. S. and Frisch A. W., 1951, Am. J. Clin. Pathol., 21:101.

Further Information

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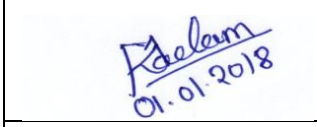		
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