Charcoal Agar

Intended Use

Charcoal Agar is used for cultivating fastidious organisms, especially *Bordetella pertussis*, for vaccine production and stock culture maintenance.

Summary and Explanation

Charcoal Agar is prepared according to the method of Mishulow, Sharpe and Cohen.[1] The authors found this medium to be an efficient substitute for Bordet-Gengou Agar in the production of *B. pertussis* vaccines.

The genus *Bordetella* consists primarily of four species: *Bordetella pertussis*, *B. parapertussis*, *B. bronchiseptica* and *B. avium;* additional species have recently been described.[2] All *Bordetella* are respiratory pathogens, residing on the mucous membranes of the respiratory tract. *B. pertussis* is the major cause of whooping cough or pertussis. *B. parapertussis* is associated with a milder form of the disease.[3] *B. bronchiseptica* is an opportunistic human pathogen associated with both respiratory and non-respiratory infections, often occurring in patients having close contact with animals.[2] *B. bronchiseptica* has not been reported to cause pertussis. There have been no reports of recovery of *B. avium* from humans.[2]

User Quality Control

Identity Specifications

Difco™ Charcoal Agar

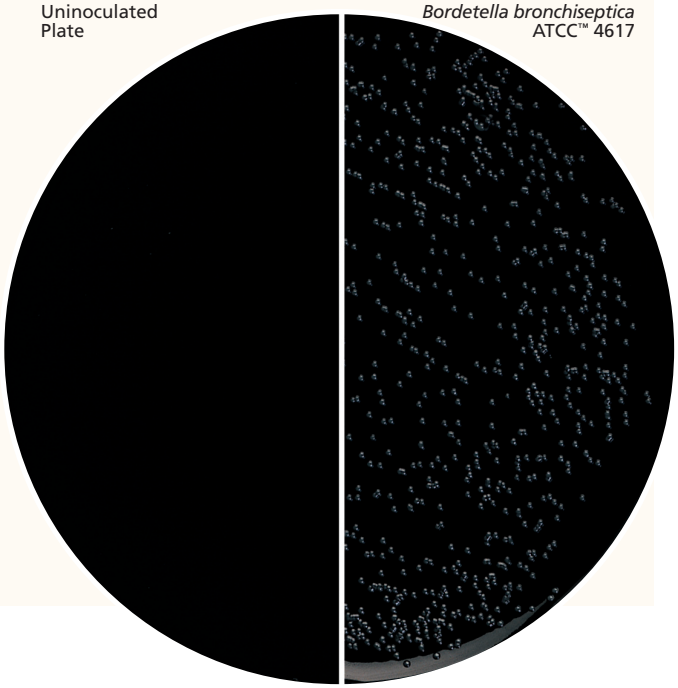
Dehydrated Appearance:	Gray, free-flowing, homogeneous.
Solution:	6.25% solution, soluble in purified water upon boiling. Solution is black, opaque with a precipitate.
Prepared Appearance:	Black, opaque.
Reaction of 6.25% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Charcoal Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bordetella bronchiseptica	4617	10^2-10^3	Good
Bordetella parapertussis	15237	10^2-10^3	Good
Bordetella pertussis	8467	10^2-10^3	Good



Charcoal Agar supplemented with Horse Blood is used for the cultivation and isolation of *Haemophilus influenzae*.[4]

Principles of the Procedure

Infusion from beef heart and peptone provide the nitrogen, carbon and amino acids in Charcoal Agar. Yeast extract is a vitamin source. Sodium chloride maintains osmotic balance. Agar is the solidifying agent. Soluble starch and Norit SG, charcoal, neutralize substances toxic to *Bordetella* species, such as fatty acids.

Formula

Difco™ Charcoal Agar

Approximate Formula* Per Liter		
Beef Heart, Infusion from 500 g	12.0	g
Peptone	10.0	g
Sodium Chloride	5.0	g
Soluble Starch	10.0	g
Yeast Extract	3.5	g
Norit SG	4.0	g
Agar	18.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 62.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Mix thoroughly during dispensing to uniformly distribute the charcoal.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion on the isolation and maintenance of fastidious microorganisms refer to the procedures described in appropriate references.[2,4,5]

Expected Results

Refer to appropriate references and procedures for results.

Limitation of the Procedure

Charcoal has a tendency to settle out of the medium. Swirl the flask gently when dispensing to obtain a uniform charcoal suspension.[4]

References

1. Mishulow, Sharpe and Cohen. 1953. Am. J. Public Health, *43*:1466.
2. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
3. Linneman and Pery. 1977. Am. J. Dis. Child. *131*:560.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol 1. Williams & Wilkins, Baltimore, Md.
5. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Charcoal Agar

Cat. No. 289410 Dehydrated – 500 g