

Procedure

1. Streak a sample of the specimen onto the surface of the agar. Make several stabs into the medium along the streak.
2. Incubate, aerobically, at 30 ± 2°C for up to 48 hours.
3. Examine for growth and the presence or absence of clear zones around colonies.
4. To determine mannitol fermentation, add a few drops of bromcresol purple to areas on the medium from which colonies have been removed. Any change in color of the indicator, compared with that of the uninoculated medium, indicates fermentation of mannitol.

Expected Results

Mannitol fermentation: Positive = change in color of the indicator to yellow.

Gelatinase activity: Positive Stone reaction = formation of clear zones around the colonies.

Any mannitol-positive, yellow or orange colonies surrounded by a clear zone are presumptively identified as *Staphylococcus*

aureus. White or nonpigmented colonies, with or without a clear zone, are probably *S. epidermidis*.

Limitations of the Procedure

1. Confirm the presumptive identification of pathogenic staphylococci with additional tests, such as coagulase activity.
2. Enterococci and/or Group D streptococci may exhibit growth on the medium and show slight mannitol fermentation. The colonies, however, are tiny and can easily be differentiated from staphylococci by Gram stain and the catalase test.³

References

1. Chapman. 1948. Food Res. 13:100.
2. Chapman. 1946. J. Bacteriol. 51:409.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ Chapman Stone Medium
 Cat. No. 211805 Dehydrated – 500 g

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*

User Quality Control				Uninoculated Plate	<i>Bordetella bronchiseptica</i> ATCC™ 4617
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		
<i>Bordetella bronchiseptica</i>	4617	10 ² -10 ³	Good		
<i>Bordetella parapertussis</i>	15237	10 ² -10 ³	Good		
<i>Bordetella pertussis</i>	8467	10 ² -10 ³	Good		



has not been reported to cause pertussis. There have been no reports of recovery of *B. avium* from humans.²

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. Hoppe. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th 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 (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Charcoal Agar

Cat. No. 289410 Dehydrated – 500 g

Chocolate II Agar • Chocolate II Agar with Bacitracin Chocolate II Agar with Pyridoxal

Intended Use

Chocolate II Agar is an improved medium for use in qualitative procedures for the isolation and cultivation of fastidious microorganisms, especially *Neisseria* and *Haemophilus* species, from a variety of clinical specimens. Media provided in divided (two-sectored) plates offer the ability to utilize the properties of two media in one plate.

Chocolate II Agar with Bacitracin is a selective medium used for the isolation of *Haemophilus* species.

Chocolate II Agar with Pyridoxal is used for the isolation of nutritionally-variant streptococci (vitamin B₆-requiring streptococci) from blood cultures.

Summary and Explanation

Carpenter and Morton described an improved medium for the isolation of the gonococcus in 24 hours.¹ The efficiency of this medium, GC Agar supplemented with hemoglobin and yeast concentrate, was demonstrated in a study of twelve media then in use for the isolation of this organism.² The medium was improved by replacing the yeast concentrate with BBL™ IsoVitalX™ Enrichment, a chemically defined supplement

developed specially to aid the growth of gonococci, although it has broad application for other microorganisms; e.g., *Haemophilus*.^{3,4} Through careful selection and pretesting of raw materials, Chocolate II prepared plated medium promotes improved growth of gonococci and *Haemophilus* species. With most strains of *N. gonorrhoeae*, visible growth on primary isolation is seen after incubation of 18-24 hours.

The isolation of fastidious organisms from specimens containing mixed flora is facilitated by selective agents. Bacitracin has been recommended for isolation of *Haemophilus* from the respiratory tract.^{5,6}

Chocolate II Agar is often used as the medium for subculture from blood culture bottles to detect the presence of bacteria in cases of septicemia. Some cases of septicemia are caused by organisms referred to as “nutritionally variant streptococci.” These organisms require certain forms of vitamin B₆, such as pyridoxal or pyridoxamine, and will not be isolated by the use of unsupplemented media.⁷ Chocolate II Agar supplemented with pyridoxal may be used for this purpose.