BBL™ Chocolate II Agar with Bacitracin
BBL™ Chocolate II Agar with Pyridoxal

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Chocolate II Agar with Bacitracin is a selective medium for the isolation of *Haemophilus* species. Chocolate II Agar with Pyridoxal is for the isolation of nutritionally variant catalase-negative, gram-positive cocci from blood cultures.

II PERFORMANCE TEST PROCEDURE

1. Spread-inoculate representative samples with 30-300 CFU/0.1 mL of the cultures listed below. Use 30-300 CFU/0.001 mL for the organisms inhibited on Chocolate II Agar with Bacitracin.
2. Incubate plates at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide.
3. Include plates of a previously tested lot of Chocolate II Agar as controls for all strains and a **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plate for *G. adiacens*.
4. Examine plates after 42–48 h for growth.
5. Expected Results

A. Chocolate II Agar with Bacitracin

Organisms	ATCC™	Recovery
**Haemophilus influenzae*	10211	Growth
Neisseria lactamica	23970	Inhibition (partial)
**Streptococcus pneumoniae*	6305	Inhibition (partial)

B. Chocolate II Agar with Pyridoxal

Organisms	ATCC	Recovery
**Granulicatella adiacens*	43205	Growth (no growth on TSA II control)
**Haemophilus influenzae*	10211	Growth
H. parainfluenzae	51505	Growth
Streptococcus pneumoniae	6305	Growth

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.2 ± 0.2 for both Chocolate II Agar with Bacitracin and Chocolate II Agar with Pyridoxal.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Chocolate II Agar is an improved medium for use in qualitative procedures for the isolation and cultivation of fastidious microorganisms.

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 catalase-negative, gram-positive cocci (vitamin B_6-requiring) from blood cultures.

V SUMMARY AND EXPLANATION

Carpenter and Morton described an improved medium for the isolation of the gonococcus in 24 h.[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 IsoVitaleX** Enrichment, a chemically defined supplement developed specially to aid the growth of gonococci, although it has broad application for other microorganisms, e.g., *Haemophilus*.[3-5] Through careful selection and pretesting of raw materials, Chocolate II prepared plated medium promotes improved growth of gonococci and *Haemophilus* species.

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.[6,7]

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 traditionally known as "nutritionally variant streptococci." More recently, these catalase-negative, gram-positive cocci have been classified as members of the genera *Abiotrophia* and *Granulicatella*.[8] These organisms require certain forms of vitamin B_6, such as pyridoxal or pyridoxamine, and will not be isolated by the use of unsupplemented media.[9] Chocolate II Agar supplemented with pyridoxal may be used for this purpose.

VI PRINCIPLES OF THE PROCEDURE

Chocolate II Agar contains an improved GC Agar base, bovine hemoglobin and **IsoVitaleX** Enrichment. The GC base contains nitrogenous nutrients in the form of casein and meat peptones, phosphate buffer to maintain pH and corn starch, which neutralizes toxic fatty acids that may be present in the agar. Hemoglobin provides X factor (hemin) for *Haemophilus* species.

IsoVitaleX Enrichment is a defined supplement which provides V factor (nicotinamide adenine dinucleotide, NAD) for *Haemophilus* species.

Chocolate II Agar with Bacitracin is a selective medium for the isolation of *Haemophilus* species from specimens containing mixed flora. Bacitracin is a polypeptide antibiotic that inhibits gram-positive bacteria and *Neisseria*.[10]

Chocolate II Agar with Pyridoxal is an enriched medium that supports the growth of nutritionally variant catalase-negative, gram-positive cocci, as well as *Haemophilus* and other fastidious microorganisms. Pyridoxal is a vitamin B_6 compound that is required for the growth of certain strains of *Abiotrophia* and *Granulicatella* (formerly of the genus *Streptococcus*).[8,9]

VII REAGENTS

Chocolate II Agar (GC II Agar with Hemoglobin and IsoVitaleX™ Enrichment)

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	7.5 g	Sodium Chloride	5.0 g
Selected Meat Peptone	7.5 g	Agar	12.0 g
Corn Starch	1.0 g	Hemoglobin	10.0 g
Dipotassium Phosphate	4.0 g	**IsoVitaleX** Enrichment	10.0 mL
Monopotassium Phosphate	1.0 g		

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

IsoVitaleX™ Enrichment

Approximate Formula* Per Liter Purified Water

Vitamin B_{12}	0.01 g	Thiamine Pyrophosphate	0.1 g
L-Glutamine	10.0 g	Ferric Nitrate	0.02 g
Adenine	1.0 g	Thiamine Hydrochloride	0.003 g
Guanine Hydrocholoride	0.03 g	L-Cysteine Hydrochloride	25.9 g
p-Aminobenzoic Acid	0.013 g	L-Cysteine	1.1 g
Nicotinamide Adenine Dinucleotide	0.25 g	Dextrose	100.0 g

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

Chocolate II Agar with Bacitracin is Chocolate II Agar modified by the addition of 16,500 units/L of bacitracin.

Chocolate II Agar with Pyridoxal is Chocolate II Agar modified by the addition of 0.01 g/L of pyridoxal.

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"[11-14] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2-8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2-8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

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

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[15,16] Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Chocolate II Agar with Bacitracin or Chocolate II Agar with Pyridoxal

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora.

Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate plates at 35 ± 2°C in an aerobic atmosphere enriched with 5–10% carbon dioxide. Examine after overnight incubation and at 24-h intervals for up to 72 h.

User Quality Control: See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After a minimum of 18 h of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Haemophilus may appear as small (1 mm), moist, pearly colonies with a characteristic "mousy" odor.

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[15-20]

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. It should be recognized that organisms generally susceptible to the antimicrobial agent in a selective medium may be completely or only partially inhibited depending upon the concentration of the agent, the characteristics of the microbial strain and the number of organisms in the inoculum. Organisms that are generally resistant to the antimicrobial agent should not be inhibited. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII AVAILABILITY

Cat. No.	Description
296271	**BBL™** Chocolate II Agar with Bacitracin, Pkg. of 20 plates
297259	**BBL™** Chocolate II Agar with Pyridoxal, Ctn. of 100 plates

XIII REFERENCES

1. Carpenter, C.M., and H.E. Morton. 1947. An improved medium for isolation of the gonococcus in 24 hours. Proc. N.Y. State Assoc. Public Health Labs. *27*:58-60.
2. Carpenter, C.M., M.A. Bucca, T.C. Buck, E.P. Casman, C.W. Christensen, E. Crowe, R. Drew, J. Hill, C.E. Lankford, H.E. Morton, L.R. Peizer, C.I. Shaw, and J.D. Thayer. 1949. Evaluation of twelve media for the isolation of the gonococcus. Am. J. Syphil. Gonorrh. Venereal Diseases *33*:164-176.
3. Power, D.A. (ed.), and P.J. McCuen. 1988. Manual of BBL products and laboratory procedures, 6th ed. Becton Dickinson Microbiology Systems, Cockeysville, Md.
4. Martin, J.E., T.E. Billings, J.F. Hackney, and J.D. Thayer. 1967. Primary isolation of *N. gonorrhoeae* with a new commercial medium. Public Health Rep. *82*:361-363.
5. Vastine, D.W., C.R. Dawson, I. Hoshiwara, C. Yonega, T. Daghfous, and M. Messadi. 1974. Comparison of media for the isolation of *Haemophilus* species from cases of seasonal conjunctivitis associated with severe endemic trachoma. Appl. Microbiol. *28*:688-690.
6. Chapin, K., and G.V. Doern. 1983. Selective media for recovery of *Haemophilus influenzae* from species contaminated with upper respiratory tract microbial flora. J. Clin. Microbiol. *17*:1163-1165.
7. Kilian, M. 2003. *Haemophilus*, p. 623-635. *In* P.R. Murray, E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken (ed.), Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
8. Ruoff, K.L. 2003. *Aerococcus*, *Abiotrophia*, and other infrequently isolated aerobic catalase-negative, gram-positive cocci, p. 434-444. *In* P.R. Murray, E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken (ed.), Manual of clinical microbiology, 8th ed; American Society for Microbiology, Washington , D.C.
9. Reimer, L.G., and L.B. Reller. 1981. Growth of nutritionally variant streptococci on common laboratory and 10 commercial blood culture media. J. Clin. Microbiol. *14*:329-332.
10. Garrod, L.P., and F. O'Grady. 1971. Antibiotics and chemotherapy, 3rd ed. Williams & Wilkins, Baltimore.
11. National Committee for Clinical Laboratory Standards. 2001. Approved Guideline M29-A2. Protection of laboratory workers from occupationally acquired infections, 2nd ed. NCCLS, Wayne, PA.
12. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. Infect. Control Hospital Epidemiol. *17*:53-80.
13. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
14. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L262, 17/10/2000, p. 0021-0045.
15. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R. H. Yolken (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
16. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey and Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
17. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.
18. MacFaddin, J.F. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore.
19. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn, Jr. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott-Raven, Philadelphia.
20. Isenberg, H.D. (ed.). 2004. Clinical microbiology procedures handbook, vol. 1, 2 and 3, 2nd ed. American Society for Microbiology, Washington, D.C.

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