



# BBL™ Chocolate II Agar (GC II Agar with Hemoglobin and IsoVitaleX™)

L007361 • Rev. 09 • November 2015

## QUALITY CONTROL PROCEDURES (Optional)

### I INTRODUCTION

Chocolate II Agar is an enriched medium for the isolation and cultivation of *Neisseria* species.

### II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
  - a. For *N. gonorrhoeae*, add 0.1 mL of a culture containing 30–300 CFU/0.1 mL to each plate and spread-inoculate using a sterile glass spreader. For all other organisms, use 10<sup>3</sup>–10<sup>4</sup>/0.1 mL and spread-inoculate.
  - b. Incubate plates at 35 ± 2 °C in an aerobic atmosphere supplemented with carbon dioxide.
  - c. Include plates of a previously tested lot of Chocolate II Agar as controls for all strains.
2. Examine plates after 18–24 and 48 h for growth.
3. Expected Results

Organisms	ATCC®	Recovery
* <i>Neisseria gonorrhoeae</i>	43069	Growth
* <i>Haemophilus influenzae</i>	10211	Growth
<i>Neisseria gonorrhoeae</i>	35201	Colonies small, opaque, grayish-white to colorless, raised, glistening and smooth
<i>Neisseria meningitidis</i>	13090	Growth
<i>Haemophilus parainfluenzae</i>	51505	Small (0.5 mm), moist, pearly colonies; “mousy” odor
<i>Streptococcus pneumoniae</i>	6305	Growth
<i>Streptococcus pyogenes</i>	19615	Colonies small to medium, white to gray, and may exhibit green discoloration of the medium

\*Recommended organism strain for User Quality Control.

**NOTE:** Must be monitored by users, according to CLSI M22-A3.

### 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.
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, especially *Neisseria* and *Haemophilus* species, from a variety of clinical specimens.

### V SUMMARY AND EXPLANATION

Carpenter and Morton described an improved medium for the isolation of the gonococcus in 24 h.<sup>1</sup> 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.<sup>2</sup> 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*.<sup>3-5</sup> 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 h.

### 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 and vitamins, amino acids, co-enzymes, dextrose, ferric ion and other factors which improve the growth of pathogenic *Neisseria*.

### 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	<b>IsoVitaleX</b> 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 <sub>12</sub> .....	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 Hydrochloride.....	0.03 g	L-Cysteine Hydrochloride.....	25.9 g
p-Aminobenzoic Acid.....	0.013 g	L-Cystine.....	1.1 g
Nicotinamide Adenine Dinucleotide.....	0.25 g	Dextrose.....	100.0 g

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

### 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"<sup>6-9</sup> 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.<sup>10,11</sup> 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

**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. Alternatively, if material is being cultured directly from a swab, proceed as follows:<sup>12</sup>

1. Roll swab directly on the medium in a large "Z" to provide adequate exposure of swab to the medium for transfer of organisms.
2. Cross-streak the "Z" pattern with a sterile wire loop, preferably in the clinic. If not done previously, cross-streaking should be done in the laboratory.
3. Place the culture as soon as possible in an aerobic environment enriched with carbon dioxide.
4. Incubate at 35 ± 2 °C and examine after overnight incubation and again after approximately 48 h.
5. Subcultures for identification of *N. gonorrhoeae* should be made within 18–24 h.

**User Quality Control:** See "Quality Control Procedures."

Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory's standard quality control procedures.

## X RESULTS

Typical colonial morphology on Chocolate II Agar is as follows:

*Haemophilus influenzae* ..... Small (1 mm), moist, pearly with a characteristic "mousy" odor  
*Neisseria gonorrhoeae* ..... Small, grayish-white to colorless, mucoid  
*Neisseria meningitidis* ..... Medium to large, blue-gray, mucoid

## XI LIMITATIONS OF THE PROCEDURE

Chocolate II Agar is an enriched medium on which pathogenic bacteria may be overgrown with undesirable or nonpathogenic bacteria.

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.<sup>10,11,13-16</sup>

## XII AVAILABILITY

**Cat. No. Description**

221169 **BD BBL™** Chocolate II Agar (GC II Agar with Hemoglobin and **IsoVitaleX™**), Pkg. of 20 plates

221267 **BD BBL™** Chocolate II Agar (GC II Agar with Hemoglobin and **IsoVitaleX™**), 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. 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. National Committee for Clinical Laboratory Standards. 2001. Approved Guideline M29-A2. Protection of laboratory workers from occupationally acquired infections, 2nd ed. NCCLS, Wayne, PA.
7. 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.
8. 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.
9. 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.
10. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R. H. Tenover (ed.). 2003. *Manual of clinical microbiology*, 8th ed. American Society for Microbiology, Washington, D.C.
11. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. *Bailey and Scott's diagnostic microbiology*, 11th ed. Mosby, Inc., St. Louis.
12. Center for Disease Control. 1975. *Criteria and techniques for the diagnosis of gonorrhea*. U.S. Public Health Service, Atlanta.
13. 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.
14. MacFaddin, J.F. 2000. *Biochemical tests for identification of medical bacteria*, 3rd ed. Lippincott Williams & Wilkins, Baltimore.
15. 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.
16. Isenberg, H.D. (ed.). 2004. *Clinical microbiology procedures handbook*, vol. 1, 2 and 3, 2nd ed. American Society for Microbiology, Washington, D.C.

Technical Information: In the United States, contact BD Technical Service and Support at 800-638-8663 or [www.bd.com/ds](http://www.bd.com/ds).

Becton, Dickinson and Company  
7 Loveton Circle  
Sparks, MD 21152 USA

ATCC is a trademark of the American Type Culture Collection.

BD, BD Logo, and all other trademarks are property of Becton, Dickinson and Company. © 2015 BD