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

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

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

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Using a 0.01 mL calibrated loop, inoculate the slant surfaces using 10^{-1} dilutions of 18- to 24-h **Trypticase™** Soy Broth cultures.
   b. Incubate tubes with loosened caps at 35 ± 2 °C in an aerobic atmosphere supplemented with carbon dioxide.
2. Examine tubes after 18 – 24 and 48 h for growth.
3. Expected Results

Organisms	ATCC®	Recovery
**Neisseria gonorrhoeae*	43069	Growth
**Haemophilus influenzae*	10211	Growth
Neisseria meningitidis	13090	Growth
Streptococcus pneumoniae	6305	Growth

*Recommended organism strain for User Quality Control.

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

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under “Product Deterioration.”
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Incubate uninoculated representative tubes at 20 – 25 °C and 30 – 35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

The Chocolate II Agar slant is an improved medium for use in qualitative procedures for 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.[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] BD improved the medium by replacing the yeast concentrate with **IsoVitaleX™** Enrichment, a chemically defined supplement developed specifically 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 tubed medium promotes improved growth of gonococci and *Haemophilus* species.

VI PRINCIPLES OF THE PROCEDURE

Chocolate II Agar contains an improved GC II 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	**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 B12	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.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2 – 8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying 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.[6,7] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Chocolate II Agar Slants

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

Test Procedure: Observe aseptic techniques.

1. Chocolate II Agar slants are primarily used for the cultivation and maintenance of pure cultures. The slants should be inoculated with a loopful of culture.
2. Place the culture as soon as possible in an aerobic environment enriched with carbon dioxide.
3. Incubate at 35 ± 2 °C and examine after overnight incubation and again after approximately 48 h.

NOTE: Subcultures for identification of *N. gonorrhoeae* should be made within 18 – 24 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 guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Typical colonial morphology on Chocolate II Agar is as follows:

Haemophilus influenzae ..... Small (1 mm), moist, pearly with characteristic “mousy” odor
Neisseria gonorrhoeae ..... Small, grayish-white to colorless, mucoid
Neisseria meningitidis ..... Medium to large, blue-gray, mucoid
Streptococcus pneumoniae ..... Small, shiny, flattened colonies which exhibit green discoloration of the medium

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.[6-8]

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Chocolate II Agar slants (GC II Agar with Hemoglobin and **IsoVitaleX**) are tested for performance characteristics. Representative samples of the lot are inoculated with 0.01 mL of a 10^{-1} dilution of 24-h **Trypticase** Soy Broth cultures of *Neisseria gonorrhoeae* (ATCC 43069), *Neisseria meningitidis* (ATCC 13090) and *Haemophilus influenzae* (ATCC 10211). The tubes, with loosened caps, are incubated at 35 ± 2 °C in an aerobic atmosphere supplemented with carbon dioxide. After 18 – 24 h incubation, growth on the slants is observed to be moderate to heavy for all organisms tested.

XIII AVAILABILITY

Cat. No.	Description
295872	**BD BBL™** Chocolate II Agar Slants (GC II Agar with Hemoglobin and **IsoVitaleX™**), Pkg. of 10 size K tubes

XIV 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.
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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. 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.
7. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
8. 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.

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