BBL™ Chocolate II Agar
(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 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 10^3–10^4/0.1 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
**Neisseria gonorrhoeae*	43069	Growth
**Haemophilus influenzae*	10211	Growth
Neisseria gonorrhoeae	35201	Colonies small, opaque, grayish-white to colorless, raised, glistening and smooth
Neisseria meningitidis	13090	Growth
Haemophilus parainfluenzae	51505	Small (0.5 mm), moist, pearly colonies; "mousy" odor
Streptococcus pneumoniae	6305	Growth
Streptococcus pyogenes	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.[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. 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	**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 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"[6-9] 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.[10,11] 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:[12]

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."

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

Typical colonial morphology on Chocolate II Agar is as follows:

Haemophilus influenzae .....Small (1mm), 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.[10,11,13-16]

XII AVAILABILITY

Cat. No.	Description
221169	**BBL™** Chocolate II Agar (GC II Agar with Hemoglobin and **IsoVitaleX™**), Pkg. of 20 plates
221882	**BBL™** Chocolate II Agar (GC II Agar with Hemoglobin and **IsoVitaleX™**), Pkg. of 30 space-saver plates
221267	**BBL™** Chocolate II Agar (GC II Agar with Hemoglobin and **IsoVitaleX™**), Ctn. of 100 plates

XIII REFERENCES

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