CHOCOLATE AGAR

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.

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™ 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*.[3,4] 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.

PRINCIPLE

Chocolate II Agar contains an improved GC Agar base (GC II Agar Base), bovine hemoglobin and IsoVitaleX Enrichment.

GC II Agar 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*.

REAGENTS (FORMULA)

Proteose Peptone	15.0	g
Corn Starch	1.0	g
Dipotassium Phosphate	4.0	g
Monopotassium Phosphate	1.0	g
Sodium Chloride	5.0	g
Agar	10.0	g
Deionized Water	1000.0	ml

PROCEDURE

Streak the specimen as soon as possible after it is received in the laboratory. Place the culture in an aerobic environment enriched with carbon dioxide. Incubate at 35 ± 2°C and examine after overnight incubation and again after approximately 48 hours. Subcultures for identification of *N. gonorrhoeae* should be made within 18-24 hours.

EXPECTED 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.

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
Haemophilus influenzae ATCC 10211	35 ± 2°C for 18-24 hours	Growth
Neisseria gonorrhoeae ATCC 43069	35 ± 2°C for 18-24 hours	Growth

ATCC® is a registered trademark of American Type Culture Collection.

BIBLIOGRAPHY

1. Carpenter and Morton. 1947. Proc. N.Y. State Assoc. Public Health Labs. 27:58.

2. Carpenter, Bucca, Buck, Casman, Christensen, Crowe, Drew, Hill, Lankford, Morton, Peizer, Shaw and Thayer. 1949. Am. J. Syphil. Gonorrh. Venereal Diseases 33:164.

3. Martin, Billings, Hackney and Thayer. 1967. Public Health Rep. 82:361

4. Vastine, Dawson, Hoshiwara, Yonega, Daghfous and Messadi. 1974. Appl. Microbiol. 28:688.

CBSA life

155-196 Innovation Drive, Winnipeg, MB, R3T 2N2, Canada

Phone: +1 (204) 269-2255 Email: info@cbsalife.com Website: https://cbsalife.com