BD BBL™ Medium Enrichment for Fastidious Microorganisms

IsoVitaleX™ Enrichment

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INTENDED USE

BD BBL™ IsoVitaleX™ Enrichment is a chemically defined supplement used as an additive to media for cultivation of nutritionally fastidious microorganisms.

SUMMARY AND EXPLANATION

Carpenter and Morton described an improved "chocolate" 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 12 media then in use for the isolation of this organism.[2] The medium was improved by replacing the yeast concentrate with BD BBL IsoVitaleX Enrichment, a chemically defined supplement developed specifically to aid the growth of gonococci, although it has broad application for other organisms, e.g., *Haemophilus*.[3,4]

Thayer and Martin developed a selective "chocolate" medium, Thayer-Martin Selective Agar, for the primary isolation of *Neisseria gonorrhoeae* and *N. meningitidis*[5] and improved it by using BD BBL IsoVitaleX Enrichment as a nutritional supplement.[3]

Since then, BD BBL IsoVitaleX Enrichment has been employed in improved media for the cultivation of pathogenic *Neisseria*, e.g., selective Modified Thayer-Martin Agar,[6] Martin-Lewis Agar,[7] and Transgrow Medium,[8] as well as supplemented GC agar (GC Agar with BD BBL IsoVitaleX Enrichment) for antimicrobial disc diffusion susceptibility testing of *N. gonorrhoeae*.[9]

PRINCIPLES OF THE PROCEDURE

BD BBL IsoVitaleX Enrichment provides V factor (nicotinamide adenine dinucleotide, NAD) for *Haemophilus* species and vitamins, amino acids, coenzymes, dextrose, ferric ions and other factors which improve the growth of pathogenic *Neisseria*.

REAGENTS

Approximate Formula* per L 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.

Each vial of BD BBL IsoVitaleX Enrichment is supplied with a vial of diluent (reconstituting solution).

Warnings and Precautions

Danger

H314 Causes severe skin burns and eye damage.

P260 Do not breathe dusts or mists. **P280** Wear protective gloves/protective clothing/eye protection/face protection. **P264** Wash thoroughly after handling. **P303+P361+P353** If on skin (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. **P305+P351+P338** If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **P310** Immediately call a POISON CENTER/doctor. **P321** Specific treatment (see on this label). **P304+P340** IF INHALED: Remove person to fresh air and keep comfortable for breathing. **P363** Wash contaminated clothing before reuse. **P301+P330+P331** If swallowed: Rinse mouth. Do NOT induce vomiting. **P405** Store locked up. **P501** Dispose of contents/container in accordance with local/regional/national/international regulations.

For Laboratory Use

This Product Contains Dry Natural Rubber.

Observe aseptic techniques in the restoration and addition of this medium enrichment.

Storage and Reconstitution Instructions

On receipt, store at 2–8 °C. After reconstitution, use immediately, or store at 2–8 °C and use within 2 weeks.

Reconstitute each lyophilized vial by aseptically transferring with a sterile syringe and needle the accompanying diluent. Shake to assure complete solution.

The expiration date applies to product in intact container stored as directed.

Product Deterioration: Some variation in the appearance of the lyophilized product may occur. This results from the lyophilization process and does not affect performance of the product.

Examine diluent and reconstituted enrichment at the time of use for evidence of contamination, evaporation, or other signs of deterioration.

PROCEDURE

Materials Provided: BD BBL IsoVitaleX Enrichment

Materials Required But Not Provided: The other ingredients and equipment required to prepare the complete medium.

Test Procedure:

Preparation of Chocolate Agar

1. Prepare a double strength base by suspending 7.2 g of GC base medium in 100 mL of purified water, using a 500 mL flask. Mix thoroughly, heat with frequent agitation and boil for about 1 min to assure complete solution of ingredients.
2. Suspend 2 g BBL Hemoglobin Powder in 100 mL purified water to make a 2% solution. (Mix 2 g of Hemoglobin Powder with 2 to 3 mL purified water until a smooth paste is achieved. Gradually add the balance of the water until the solution is homogeneous. If larger volumes are required, use the same method, maintaining the same ratio of Hemoglobin to purified water.) Alternatively, use BBL Hemoglobin Solution 2% warmed to approximately 50 °C.
3. Autoclave separately the GC base medium and Hemoglobin solution (if prepared from the powder) at 121 °C for 15 min.
4. Cool the autoclaved solutions to approximately 50 °C.
5. Reconstitute BD BBL IsoVitaleX Enrichment, 2 mL (see “Storage and Reconstitution Instructions”).
6. Aseptically add the 100 mL of Hemoglobin and 2 mL of BD BBL IsoVitaleX Enrichment to the 100 mL of GC base medium.
7. Mix gently but thoroughly and distribute into sterile Petri dishes or other sterile containers.

The BD BBL IsoVitaleX Enrichment 10 mL is used similarly, by adding the reconstituted contents of one vial to 500 mL of autoclaved and cooled (approximately 50 °C) GC base medium (36.0 g of the base in 500 mL purified water to make a double strength base) and 500 mL of autoclaved 2% Hemoglobin solution (approximately 50 °C).

Preparation of Selective Media: For the preparation of Thayer-Martin Selective Agar, Modified Thayer-Martin Agar and Transgrow Medium, see the product insert for BBL V-C-N and V-C-N-T Inhibitors. For the preparation of Martin-Lewis Agar, see the product insert for V-C-A and V-C-A-T Inhibitors.

Preparation of Supplemented GC Agar[9]: Prepare single strength GC base medium and autoclave at 121 °C for 15 min. Cool to approximately 50 °C. Reconstitute BD BBL IsoVitaleX Enrichment (see “Storage and Reconsitution Instructions”). Add reconstituted BD BBL IsoVitaleX Enrichment to yield a final concentration of 1%.

User Quality Control: Examine lyophilized and reconstituted enrichment for signs of deterioration as noted under “Product Deterioration”.

Check performance of the complete medium with pure cultures of stable control organisms producing known desired reactions. The following cultures are recommended:

Chocolate Agar (aerobic atmosphere supplemented with CO_2; 35 ± 2 °C; 18–24 h):

Neisseria gonorrhoeae	ATCC® 43069	Growth
Haemophilus influenzae	ATCC 10211	Growth

GC medium with BD BBL IsoVitaleX Enrichment (5–7% CO_2; 35 °C; 20–24 h):

Neisseria gonorrhoeae	ATCC 49226	Growth

(For demonstrating suitability for use in antimicrobial disc diffusion susceptibility testing, refer to the reference.[9])

LIMITATIONS OF THE PROCEDURE

Chocolate Agar is an enriched medium in which pathogenic bacteria may be overgrown with undesirable or nonpathogenic bacteria. A medium selective for pathogenic *Neisseria* should be used in conjunction with Chocolate Agar when bacteria such as *N. gonorrhoeae* and *N. meningitidis* are suspected in clinical specimens.

AVAILABILITY

Cat. No.	Description
211875	BD BBL™ IsoVitaleX™ Enrichment, 5 vials each of enrichment and diluent (each reconstitutes to 2 mL)
211876	BD BBL™ IsoVitaleX™ Enrichment, 5 vials each of enrichment and diluent (each reconstitutes to 10 mL)

REFERENCES

1. Carpenter, C.M., and H.E. Morton. 1947. An improved medium for isolation of 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. 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.
4. 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.
5. Thayer, J.D., and J.E. Martin, Jr. 1966. Improved medium selective for cultivation of *N. gonorrhoeae* and *N. meningitidis*. Public Health Rep. *81*:559–562.
6. Martin, J.E., J.H. Armstrong, and P.B. Smith. 1974. New system for cultivation of *Neisseria gonorrhoeae*. Appl. Microbiol. *27*:802–805.
7. Martin, J.E. Jr., and J.S. Lewis. 1977. Anisomycin: improved antimycotic activity in modified Thayer-Martin medium. Public Health Lab. *35*:53–62.
8. Martin, J.E. Jr., and A. Lester. 1971. Transgrow, a medium for transport and growth of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. HSMHA Health Rep. *86*:30–33.
9. National Committee for Clinical Laboratory Standards.1993. Approved standard: M2–A5. Performance standards for antimicrobial disk susceptibility tests, 5th ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.

Technical Information: In the United States contact BD Technical Service and Support at 1.800.638.8663 or www.bd.com.

Change History

Revision/Date	Section	Change Summary
(03) 2019-02	All	Updated BD branding. Updated trademark line.
	Warnings and Precautions	Updated the “Warnings and Precautions” section with new GHS requirements for P codes.

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