INTENDED USE
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 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. meningitidis5 and improved it by using IsoVitaleX Enrichment as a nutritional supplement.3 Since then, 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 IsoVitaleX Enrichment) for antimicrobial disc diffusion susceptibility testing of N. gonorrhoeae.9

PRINCIPLES OF THE PROCEDURE
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

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12</td>
<td>0.01 g</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Adenine</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Guanine Hydrochloride</td>
<td>0.03 g</td>
</tr>
<tr>
<td>p-Aminobenzoic Acid</td>
<td>0.013 g</td>
</tr>
<tr>
<td>Nicotinamide Adenine Dinucleotide</td>
<td>0.25 g</td>
</tr>
<tr>
<td>Thiamine Pyrophosphate</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Ferric Nitrate</td>
<td>0.02 g</td>
</tr>
<tr>
<td>Thiamine Hydrochloride</td>
<td>0.003 g</td>
</tr>
<tr>
<td>L-Cysteine Hydrochloride</td>
<td>25.9 g</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>1.1 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>100.0 g</td>
</tr>
</tbody>
</table>

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

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

Warnings and Precautions

Danger

H314 Causes severe skin burns and eye damage.

P280 Wear protective gloves/protective clothing/eye protection/face protection. 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. P304+P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing. 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.

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

Materials Provided: 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 BBL IsoVitaleX Enrichment, 2 mL (see “Storage and Reconstitution Instructions”).

6. Aseptically add the 100 mL of Hemoglobin and 2 mL of 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 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: Prepare single strength GC base medium and autoclave at 121 °C for 15 min. Cool to approximately 50 °C. Reconstitute BBL IsoVitaleX Enrichment (see “Storage and Reconstitution Instructions”). Add reconstituted 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 CO₂; 35 ± 2 °C; 18 – 24 h):

- Neisseria gonorrhoeae ATCC® 43069 Growth
- Haemophilus influenzae ATCC 10211 Growth

GC medium with IsoVitaleX Enrichment (5 – 7% CO₂; 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

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