

GC Medium Base • Supplement B • Supplement VX IsoVitaleX™ Enrichment • Hemoglobin • VCN Inhibitor VCNT Inhibitor • VCA Inhibitor • VCAT

Inhibitor

Intended Use

Difco™ GC Medium Base is used with various additives in isolating and cultivating *Neisseria gonorrhoeae* and other fastidious microorganisms.

Difco Supplement B with Reconstituting Fluid B, Difco Supplement VX with Reconstituting Fluid VX and BBL™ IsoVitaleX™ Enrichment with Rehydrating Fluid are used for supplementing media to culture fastidious microorganisms, particularly *Neisseria gonorrhoeae* and *Haemophilus influenzae*.

BBL Hemoglobin products are used in preparing microbiological culture media.

BBL VCN Inhibitor, VCNT Inhibitor, VCA Inhibitor and VCAT Inhibitor are lyophilized preparations containing inhibitory agents to be used in selective media for culturing *N. gonorrhoeae* and *N. meningitidis*.

Summary and Explanation

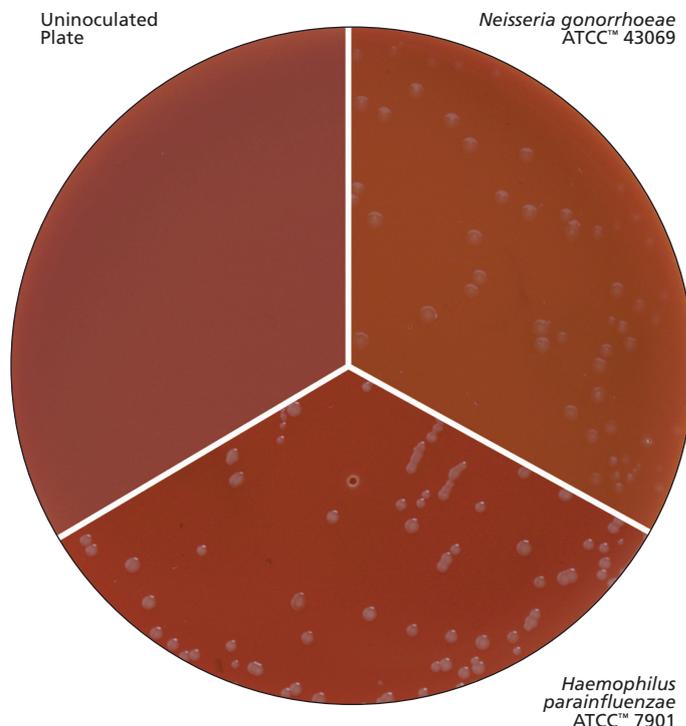
In 1945, Johnston¹ described a medium that could successfully produce colonies of *N. gonorrhoeae* in 24 rather than 48 hours. The accelerated growth rates were primarily due to the decreased agar content (solidity) of the medium. GC Medium Base was introduced in 1947 with reduced agar content. While investigating the growth rate of some gonococcal strains, a medium containing the growth factors glutamine and cocarboxylase was found to improve recovery.^{2,3} From this discovery, Supplement B, a yeast concentrate, was developed. In a comparative study⁴ of 12 different media, an enriched chocolate agar prepared with GC Medium Base, Hemoglobin and Supplement B proved superior for isolating *N. gonorrhoeae*. Difco VX Supplement and BBL™ IsoVitaleX™ Enrichment are chemically defined supplements developed to replace the yeast concentrate additive.

Difco Supplement B is a yeast concentrate for use in supplementing media for growth of microorganisms with exacting nutritional requirements. It is recommended for use in the preparation of chocolate agar described by Christensen and Schoenlein.⁵

Difco Supplement VX and BBL™ IsoVitaleX™ Enrichment are lyophilized concentrates. These supplements are recommended for enriching GC Agar media, Proteose No. 3 Agar and the selective agars for the isolation of pathogenic *Neisseria*.

Hemoglobin, an autoclavable preparation of beef blood, provides hemin, which is required by *Haemophilus* species and enhances growth of *Neisseria* species.

In 1964, Thayer and Martin⁶ formulated a selective medium



incorporating the antibiotics polymyxin B and ristocetin into GC Agar with added hemoglobin and Supplement B. Thayer and Martin⁷ improved their medium by replacing the two original antibiotics with a new antimicrobial solution of colistin, vancomycin and nystatin (VCN). In 1971, Martin and Lester⁸ improved the new Thayer-Martin medium by incorporating an additional antibiotic, trimethoprim lactate (T), into the formulation (VCNT). This improved medium is called Modified Thayer-Martin (MTM) Medium.⁹ VCN and VCNT are used in the preparation of Thayer-Martin and Modified Thayer-Martin agars, respectively.

Martin and Lewis¹⁰ further improved the selectivity of MTM by increasing the concentration of vancomycin from 3.0 µg/mL to 4.0 µg/mL for greater inhibition of gram-positive bacteria and replacing nystatin with anisomycin (VCA/VCAT) for greater inhibition of yeasts; this is known as Martin-Lewis (ML) Agar Medium. Transgrow Medium bottles and Gono-Pak and JEMBEC™* plates are chocolate agar-based transport medium systems that can incorporate these formulations.¹¹

*JEMBEC is a trademark of Miles Scientific.

Principles of the Procedure

Peptones provide nitrogen, vitamins and amino acids. Corn starch absorbs any toxic metabolites that are produced; dibasic and monobasic potassium phosphates buffer the medium. Sodium chloride maintains osmotic balance. Agar is the solidifying agent.

Chocolate Agar is prepared from GC agar medium base with the addition of 2% Hemoglobin. Hemoglobin provides hemin (X factor) required for growth of *Haemophilus* and enhanced growth of *Neisseria*.

The growth rate of *Neisseria* and *Haemophilus* is improved with the addition of 1% nutritive enrichment, providing the growth factors glutamine and cocarboxylase. Supplement B contains yeast concentrate, glutamine, coenzyme (V factor), cocarboxylase, hematin (X factor) and growth factors. Supplement VX is a defined lyophilized concentrate of essential growth factors; i.e., vitamins, amino acids, coenzymes, dextrose and other factors to improve the growth of *Haemophilus* and *Neisseria* species. IsoVitaleX Enrichment provides V factor (nicotinamide adenine dinucleotide, NAD) for *Haemophilus* species and vitamins, amino acids, coenzymes, dextrose, ferric ion and other factors which improve the growth of pathogenic *Neisseria*.

Antimicrobial agents are used as inhibitors in the selective media, Thayer-Martin, Modified Thayer-Martin and Martin Lewis agars and Transgrow Medium. Colistin inhibits gram-negative bacteria, vancomycin inhibits gram-positive contaminants, nystatin suppresses the growth of yeasts, anisomycin provides improved inhibition of *Candida albicans*, an organism that has been shown to inhibit *N. gonorrhoeae*,^{12,13} and trimethoprim lactate suppresses the swarming of *Proteus* species.

Formulae

Difco™ GC Medium Base

| Approximate Formula* Per Liter | |
|--------------------------------|--------|
| Proteose Peptone No. 3..... | 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 |

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

BBL™ Hemoglobin

An autoclavable preparation of beef blood. The 2% solution is ready for use in the preparation of media for the cultivation of fastidious organisms.

Difco™ Supplement B

Processed to preserve both the thermolabile and thermostable growth accessory factors of fresh yeast, it contains glutamine, coenzyme (V factor), cocarboxylase and other growth factors, as well as hematin (X factor).

Difco™ Supplement VX

| Approximate Formula* Per 10 mL Vial | |
|---|----------|
| Adenine Sulfate..... | 10.0 mg |
| p-Aminobenzoic Acid..... | 0.25 mg |
| Cocarboxylase..... | 2.0 mg |
| L-Cysteine HCl..... | 259.0 mg |
| L-Cystine..... | 11.0 mg |
| Nicotinamide Adenine Dinucleotide..... | 3.5 mg |
| Ferric Citrate..... | 0.3 mg |
| L-Glutamine..... | 200.0 mg |
| Guanine HCl..... | 0.3 mg |
| Thiamine HCl..... | 0.06mg |
| Vitamin B ₁₂ (Cyanocobalamin)..... | 0.2 mg |
| Dextrose..... | 1.0 g |

BBL™ IsoVitaleX™ Enrichment

| Approximate Formula* Per Liter | |
|--|---------|
| Adenine..... | 1.0 g |
| p-Aminobenzoic Acid..... | 13.0 mg |
| L-Cysteine Hydrochloride..... | 25.9 g |
| L-Cystine..... | 1.1 g |
| Nicotinamide Adenine Dinucleotide..... | 0.25 g |
| Ferric Nitrate..... | 0.02 g |
| L-Glutamine..... | 10.0 g |
| Guanine Hydrochloride..... | 0.03 g |
| Thiamine Hydrochloride..... | 3.0 mg |
| Vitamin B ₁₂ | 0.01 g |
| Dextrose..... | 100.0 g |
| Thiamine Pyrophosphate..... | 0.1 g |

BBL™ VCN Inhibitor

| Formula Per 1 mL Vial | |
|-----------------------|-------------|
| Vancomycin..... | 300 µg |
| Colistin..... | 750 µg |
| Nystatin..... | 1,250 units |

BBL™ VCNT Inhibitor

| Formula Per 1 mL Vial | |
|---------------------------|-------------|
| Vancomycin..... | 300 µg |
| Colistin..... | 750 µg |
| Nystatin..... | 1,250 units |
| Trimethoprim Lactate..... | 500 µg |

BBL™ VCA Inhibitor

| Formula Per 1 mL Vial | |
|-----------------------|--------|
| Vancomycin..... | 400 µg |
| Colistin..... | 750 µg |
| Anisomycin..... | 2.0 mg |

User Quality Control

Identity Specifications

Difco™ GC Medium Base

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 3.6% solution, soluble in purified water upon boiling. Solution is light to medium amber, opalescent, may have a slight precipitate, "ground glass" appearance.

Prepared Appearance: With Hemoglobin and Supplement, chocolate brown, opaque.

Reaction of 3.6% Solution at 25°C: pH 7.2 ± 0.2

Difco™ Supplement B with Reconstituting Fluid

Lyophilized Appearance: Tan to reddish brown, lyophilized powder or cake.

Rehydrated Appearance: Medium to dark amber, very to slightly opalescent.

Reconstituting Fluid Appearance: Colorless, clear liquid.

Difco™ Supplement VX with Reconstituting Fluid

Lyophilized Appearance: Pink powder or cake.

Rehydrated Appearance: Pink, clear.

Reconstituting Fluid Appearance: Colorless, clear solution.

BBL™ Hemoglobin Solution 2%

Solution Appearance: Dark chocolate brown with fine, very dark brown sediment, opaque.

BBL™ Hemoglobin, Bovine, Freeze-Dried

Dehydrated Appearance: Coarse powder to crystals, free of extraneous material, medium to medium dark brown to red brown to black brown.

Solution: 2% solution, soluble in purified water.

Solution Appearance: 2% solution, medium to dark medium, brown to red brown to black brown, opaque.

BBL™ IsoVitaleX™ Enrichment with Diluent

Lyophilized Appearance: Pink cake.

Rehydrated Appearance: Light pink to pink tan, clear to trace hazy.

Diluent Appearance: Colorless, clear solution.

BBL™ VCN Inhibitor

Lyophilized Appearance: Pale yellow, dry cake.

Rehydrated Appearance: Trace yellow-green and hazy containing a moderate amount of minute yellow particles.

BBL™ VCNT Inhibitor

Lyophilized Appearance: Pale green, trace yellow.

Rehydrated Appearance: Trace yellow, hazy, contains moderate amount of minute yellow particles.

BBL™ VCA Inhibitor

Lyophilized Appearance: White to off-white.

Rehydrated Appearance: Clear and colorless.

BBL™ VCAT Inhibitor

Lyophilized Appearance: White to off-white, complete and dry, with scum-like disk evident.

Rehydrated Appearance: Colorless, clear to moderately hazy.

Cultural Response

Difco™ GC Medium Base, Hemoglobin and Supplement B or Supplement VX or IsoVitaleX™ Enrichment

Prepare Chocolate Agar with GC Medium Base per label directions. Inoculate and incubate at 35 ± 2°C under 3-5% CO₂ for 18-48 hours.

| ORGANISM | ATCC™ | INOCULUM CFU | RECOVERY |
|-------------------------------|-------|--------------|----------|
| <i>Haemophilus influenzae</i> | 10211 | 30-300 | Good |
| <i>Neisseria gonorrhoeae</i> | 43069 | 30-300 | Good |

Difco™ GC Medium Base, Hemoglobin and Supplement B or Supplement VX or IsoVitaleX™ Enrichment and VCN Inhibitor or VCNT Inhibitor

Prepare Thayer-Martin Medium or Modified Thayer-Martin Medium with GC Medium Base per label directions, supplemented with VCN or VCNT Inhibitor, respectively. Inoculate and incubate at 35 ± 2°C under 3-5% CO₂ for 18-48 hours.

| ORGANISM | ATCC™ | INOCULUM CFU | RECOVERY |
|-----------------------------------|-------|--------------|-------------------------------|
| <i>Candida albicans</i> * | 60193 | 30-300 | Partial inhibition |
| <i>Neisseria gonorrhoeae</i> | 43069 | 30-300 | Good |
| <i>Neisseria meningitidis</i> * | 13090 | 30-300 | Good |
| <i>Proteus mirabilis</i> * | 43071 | 30-300 | Partial inhibition |
| <i>Staphylococcus epidermidis</i> | 12228 | 30-300 | Marked to complete inhibition |

*Only used on VCNT-containing medium.

Difco™ GC Medium Base, Hemoglobin and Supplement B or Supplement VX or IsoVitaleX™ Enrichment and VCA Inhibitor or VCAT Inhibitor

Prepare Martin-Lewis Agar with GC Medium Base according to label directions, supplemented with VCA Inhibitor or VCAT Inhibitor. Inoculate and incubate at 35 ± 2°C under 3-5% CO₂ for 48 hours.

| ORGANISM | ATCC™ | INOCULUM CFU | RECOVERY |
|-----------------------------------|-------|--------------|--------------------|
| <i>Candida albicans</i> | 60193 | 30-300 | Partial inhibition |
| <i>Neisseria gonorrhoeae</i> | 43069 | 30-300 | Good |
| <i>Neisseria meningitidis</i> * | 13090 | 30-300 | Good |
| <i>Proteus mirabilis</i> * | 43071 | 30-300 | Partial inhibition |
| <i>Staphylococcus epidermidis</i> | 12228 | 30-300 | Partial inhibition |

*Only used on VCAT-containing medium.

BBL™ VCAT Inhibitor

Formula Per 1 mL Vial

| | | |
|----------------------------|-----|----|
| Vancomycin | 400 | µg |
| Colistin | 750 | µg |
| Anisomycin | 2.0 | mg |
| Trimethoprim Lactate | 500 | µg |

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

Directions for Preparation from Dehydrated Product

Difco™ Supplement B Difco™ Supplement VX

1. Aseptically rehydrate Supplement B and Supplement VX with 10 mL or 100 mL of the corresponding Reconstituting Fluid, as appropriate.
2. Rotate the vial to dissolve completely.

BBL™ IsoVitaleX™ Enrichment

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

BBL™ Hemoglobin

1. Dissolve 10 g of Hemoglobin powder for each L of medium desired in 1/2 volume of cold purified water.
2. Autoclave solution at 121°C for 15 minutes.
3. Cool to 45-50°C and combine with 1/2 volume of autoclaved agar base solution (such as GC agar medium base).
4. Mix thoroughly before dispensing into appropriate containers.

BBL™ Hemoglobin 2%

Shake the bottle to resuspend any sedimented hemoglobin before use.

BBL™ VCN Inhibitor, VCNT Inhibitor, VCA Inhibitor, VCAT Inhibitor

1. Restore each lyophilized vial by aseptically adding the appropriate amount of sterile purified water, as indicated on the label.
2. Rotate the vial to dissolve completely.

Chocolate Agar

1. Suspend 7.2 g of GC base medium in 100 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 100 mL Hemoglobin Solution 2%.
5. Aseptically add 2 mL Supplement B or Supplement VX or IsoVitaleX Enrichment.
6. Dispense into sterile Petri dishes or tubes as desired.
7. Test samples of the finished product for performance using stable, typical control cultures.

Thayer-Martin Agar

1. Suspend 7.2 g of GC base medium in 100 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 100 mL Hemoglobin Solution 2%.
5. Aseptically add 2 mL Supplement B or Supplement VX or IsoVitaleX Enrichment.
6. Aseptically add 2 mL rehydrated VCN Inhibitor to the medium.
7. Dispense into sterile Petri dishes.
8. Test samples of the finished product for performance using stable, typical control cultures.

Modified Thayer-Martin Medium

1. Suspend 7.2 g of GC base medium in 100 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 100 mL Hemoglobin Solution 2% to the medium.
5. Aseptically add 2 mL IsoVitaleX Enrichment.
6. Aseptically add 2 mL rehydrated VCNT Inhibitor to the medium.
7. Dispense into sterile Petri dishes.
8. Test samples of the finished product for performance using stable, typical control cultures.

Martin-Lewis Agar

1. Suspend 7.2 g of GC base medium in 100 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 100 mL Hemoglobin Solution 2%.
5. Aseptically add 2 mL IsoVitaleX Enrichment.
6. Aseptically add 2 mL rehydrated VCAT Inhibitor to the medium.
7. Dispense into sterile Petri dishes.
8. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion on the isolation and identification of *Neisseria* and *Haemophilus* species, consult the procedures outlined in the references.¹⁴⁻¹⁶

Expected Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. GC Medium Base is intended for use with supplementation. Although certain diagnostic tests may be performed directly on the medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.
2. Improper specimen collection, environment, temperature, CO₂ level, moisture and pH can adversely affect the growth and viability of the organisms.
3. Inactivation or deterioration of antibiotics in selective media may allow growth of contaminants.
4. GC Medium Base has sufficient buffering capacity to offset the very low pH of the small amount of nutritive enrichments added. However, the pH of some media may have to be adjusted with 1% NaOH after the addition of these enrichments.

References

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5. Christensen and Schoenlein. 1947. Annu. Meet. Calif. Public Health Assoc.
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Availability

Difco™ GC Medium Base

| | | |
|----------|--------|--------------------|
| Cat. No. | 228950 | Dehydrated – 500 g |
| | 228920 | Dehydrated – 2 kg |
| | 228930 | Dehydrated – 10 kg |

BBL™ Hemoglobin Solution 2%

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|----------|--------|--------------|
| Cat. No. | 211874 | 10 × 100 mL* |
|----------|--------|--------------|

BBL™ Hemoglobin, Bovine, Freeze-Dried

| | | |
|----------|--------|--------------------|
| Cat. No. | 212392 | Dehydrated – 500 g |
|----------|--------|--------------------|

Difco™ Supplement B

| | | |
|----------|--------|--|
| Cat. No. | 227610 | Lyophilized – 6 × 10 mL with Reconstituting Fluid* |
| | 227620 | Lyophilized – 100 mL with Reconstituting Fluid* |

Difco™ Supplement VX

| | | |
|----------|--------|--|
| Cat. No. | 233541 | Lyophilized – 6 × 10 mL with Reconstituting Fluid* |
| | 233542 | Lyophilized – 100 mL with Reconstituting Fluid* |

BBL™ IsoVitalEx™ Enrichment

| | | |
|----------|--------|---------------------------------------|
| Cat. No. | 211875 | Lyophilized – 5 × 2 mL with Diluent* |
| | 211876 | Lyophilized – 5 × 10 mL with Diluent* |

BBL™ VCN Inhibitor

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|----------|--------|----------------------------|
| Cat. No. | 212227 | Lyophilized – 10 × 2 mL** |
| | 212228 | Lyophilized – 10 × 10 mL** |

BBL™ VCNT Inhibitor

| | | |
|----------|--------|----------------------------|
| Cat. No. | 212408 | Lyophilized – 10 × 10 mL** |
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BBL™ VCA Inhibitor

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|----------|--------|----------------------------|
| Cat. No. | 212269 | Lyophilized – 10 × 10 mL** |
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BBL™ VCAT Inhibitor

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|----------|--------|----------------------------|
| Cat. No. | 212404 | Lyophilized – 10 × 10 mL** |
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*Store at 2-8°C.

**Store at -20° to + 8°C.