BD BBL™ Medium Supplement for the Selection of Pathogenic *Neisseria*

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INTENDED USE

V-C-A Inhibitor is an antibiotic mixture of vancomycin, colistin and anisomycin which is incorporated into culture media to permit the isolation of pathogenic *Neisseria* by inhibiting contaminating flora.

V-C-A-T Inhibitor is V-C-A Inhibitor plus trimethoprim to improve recovery of pathogenic *Neisseria* by increasing the selectivity of isolation media.

SUMMARY AND EXPLANATION

V-C-A Inhibitor was developed as an improved inhibitory supplement relative to the V-C-N (Vancomycin-Colistin-Nystatin) Inhibitor employed in Thayer-Martin-type selective media for the isolation of *Neisseria gonorrhoeae* and *N. meningitidis*.[1-4]

It is employed in selective media, e.g., Martin-Lewis Agar,[5] which is Chocolate Agar (**BBL**™ GC Agar Base or GC II Agar Base, Hemoglobin and **IsoVitaleX**™ Enrichment, a chemically defined supplement developed specifically to promote the growth of *N. gonorrhoeae*) supplemented with additional dextrose, V-C-A inhibitor and trimethoprim lactate, to suppress the swarming of *Proteus* species.[6] Because of its improved performance, it is particularly recommended for the isolation of pathogenic *Neisseria*.[7]

V-C-A-T Inhibitor may be used similarly as the V-C-A Inhibitor except that trimethoprim lactate has been included in the antibiotic mixture and the addition of the antibiotic separately is not required.

PRINCIPLES OF THE PROCEDURE

The V-C-A Inhibitor contains vancomycin to inhibit gram-positive contaminants, colistin to inhibit gram-negative bacteria, including *Pseudomonas* species, and anisomycin to inhibit the growth of yeasts. V-C-A-T Inhibitor contains the above antibiotic mixture plus trimethoprim lactate, which inhibits *Proteus* species.

The reformulation V-C-N Inhibitor and V-C-N-T Inhibitor to V-C-A Inhibitor and V-C-A-T Inhibitor has resulted in the development of an improved medium, Martin-Lewis Agar, as compared to Modified Thayer-Martin (MTM) Agar and earlier formulations developed for the selective isolation of pathogenic *Neiserria*.[1] In Martin-Lewis Agar, the concentration of vancomycin is increased from 3.0 to 4.0 mcg/mL for greater inhibition of gram-positive bacteria, and anisomycin is substituted for nystatin, which was relatively ineffective at the recommended concentration of 12.5 µ/mL,[8] for improved inhibition of *Candida albicans*.

The failure of *Candida* to be suppressed by the older formulations was of concern because gonococci are inhibited in the presence of *Candida albicans*.[9-10] Anisomycin is readily soluble in water, possesses a high degree of fungicidal activity against *Candida*, while not inhibiting gonococci, and is relatively stable in prepared culture media.[5]

REAGENTS

Formulae:

V-C-A Inhibitor

Approximate Formula Per 1 mL Restored Solution:

Vancomycin	400 mcg
Colistin	750 mcg
Anisomycin	2.0 mg

V-C-A -T Inhibitor

Approximate Formula Per 1 mL Restored Solution:

Vancomycin	400 mcg
Colistin	750 mcg
Anisomycin	2.0 mg
Trimethoprim Lactate	500 mcg

Warnings and Precautions

For Labotatory Use

This Product contains Dry Natural Rubber.

V-C-A Inhibitor and V-C-A-T Inhibitor are for use in culture media and not for use in human or animal therapy.

Observe aseptic techniques in the restoration and addition of these media supplements.

Read directions for use.

Danger

H301 Toxic if swallowed.

P264 Wash thoroughly after handling. **P301+P310** IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. **P405** Store locked up. **P501** Dispose of contents/container in accordance with local/regional/national/international regulations.

Storage Instructions and Restoration: On receipt, store at -20 to +8 °C. After restoration, use immediately or store below -20 °C and use within two weeks. Avoid repeated freezing and thawing.

Restore each lyophilized vial by aseptically adding with a sterile syringe and needle 10 mL of sterile Purified Water.

The expiration date applies to product in intact container stored as directed. Do not open until ready to use.

Product Deterioration: Examine restored reagents at the time of use for evidence of contamination, evaporation or other signs of deterioration.

PROCEDURE

Material Provided: Depending upon which product is ordered, one of the medium supplements listed above is provided.

Materials Not Provided: Ancillary culture media, reagents, quality control cultures and laboratory equipment as required for this procedure.

Instructions

Preparation of Martin-Lewis Agar

1. Prepare a double-strength base by suspending 36.0 g of **BBL** GC Agar Base or GC II Agar Base in 500 mL of Purified Water. Mix thoroughly. Heat with frequent agitation and boil for about one min. to assure complete solution of ingredients.
2. Suspend 10 g of Hemoglobin Powder in 500 mL Purified Water to make a 2% solution. (Mix 10 g of Hemoglobin Powder with 10 to 15 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, the same method may be used, maintaining the same ratio of Hemoglobin to Purified Water.) Alternatively, use Hemoglobin Solution 2% warmed to approximately 50 °C.
3. Sterilize the GC Agar Base or GC II Agar Base and Hemoglobin solution, if prepared from the powder, by autoclaving at 121 °C for 15 min.
4. Cool the sterile solution to approximately 50 °C.
5. Prepare a sterile 25% dextrose solution.
6. Restore **BBL IsoVitaleX** Enrichment, 10 mL (see insert for directions).
7. Restore V-C-A Inhibitor; see “Storage Instructions and Restoration.”
8. Restore trimethoprim lactate (Burroughs-Wellcome) according to the manufacturer’s directions.
9. Aseptically add the 500 mL of sterile, cooled GC Agar Base or GC II Agar Base to the 500 mL of Hemoglobin, 6 mL of the 25% dextrose solution,* 10 mL of **IsoVitaleX** Enrichment, 10 mL of V-C-A Inhibitor and sufficient trimethoprim lactate solution, based on stated purity, to give a final concentration of 5 mcg/mL in the medium.
10. Mix gently but thoroughly and distribute into sterile Petri dishes or other sterile containers.

V-C-A-T Inhibitor is used similarly, with the exception of adding the trimethoprim lactate which is included in the antibiotic mixture.

* In the **BBL** formulation for Martin-Lewis Agar the extra dextrose has been eliminated for improved growth of *N. gonorrhoeae.*

Preparation of Martin-Lewis Agar in Bottles

1. Add extra agar to the GC Agar Base or GC II Agar Base to bring the final agar concentration to 2% and sterilize by autoclaving at 121 °C for 15 min.
2. After mixing the sterile, cooled (approximately 50 °C) GC Agar Base or GC II Agar Base, sterile and cooled Hemoglobin Solution, **IsoVitaleX** Enrichment and V-C-N Inhibitor plus trimethoprim lactate solution or V-C-A-T inhibitor, add 6 mL of sterile 25% dextrose solution, if desired, to each Liter of medium.
3. Aseptically dispense into horizontally positioned sterile 1 oz. prescription bottles (8- to 10-mL volumes) and loosely apply rubber-lined screw caps.
4. After the medium has cooled and solidified in the bottles, introduce a carbon dioxide atmosphere into the bottles by placing a group of bottles in a vacuum chamber, exhaust the air with a vacuum pump (15 lb negative pressure) and refill the chamber with a filtered mixture of 10% CO_2-90% air until the chamber is at 5 lb positive pressure; repeat this step 3 times. After the third gassing, leave the chamber at positive pressure for 2 to 3 h before returning it to atmospheric pressure.
5. Upon opening the chamber, tighten the screw caps to make an airtight seal.

USER QUALITY CONTROL

1. Examine lyophilized and restored supplement for signs of deterioration as noted under “Product Deterioration.”
2. Check performance of the finished medium by inoculation with pure cultures of stable control organisms, producing known, desired reactions. The following Cultures are recommended:

Martin-Lewis Agar

Neisseria gonorrhoeae ATCC® 43069	Growth
Staphylococcus epidermidis ATCC 12228	Inhibition (partial)
Candida albicans ATCC 60193	Inhibition (partial)
Proteus mirabilis ATCC 43071	Inhibition (partial)

RESULTS

Typical colonial morphology is as follows:

Neisseria gonorrhoeae	Small, grayish-white to colorless, mucoid
Neisseria meningitidis	Medium to large, blue-gray, mucoid

LIMITATIONS OF THE PROCEDURE

Media into which V-C-A Inhibitor and V-C-A-T Inhibitor are incorporated are selective media that may inhibit other pathogenic bacteria, e.g., *Haemophilus*. Also the existence of strains of *N. gonorrhoeae* inhibited by vancomycin and trimethoprim lactate have to be reported.[11,12] It is recommended that Chocolate Agar and blood agar plates be used in conjunction with selective media to aid in the isolation of other pathogens that may be present in the specimen.

While “saprophytic” *Neisseria* are generally suppressed by this type of selective media, the occasional recovery of *N. lactamica* on Thayer-Martin-type media has been reported.[13]

Some strains of *Capnocytophaga* species may grow on these selective media when inoculated with oropharyngeal specimens.[14]

Since there is no such entity as a perfect medium, some strains of microorganisms are encountered that grow poorly on a particular medium, the nature of the specimens or samples themselves and the physiologic state of the organisms on isolation can influence recovery of desired species, as well as modify the effects of inhibitory characteristics of a selective medium for undesired species.

It should be noted that situations are relatively rare when a single medium will suffice both for detection and for enumeration of specific microorganisms. Each selective medium represents a compromise in that selective agents, while being inhibitory to many undesired species, may also be somewhat inhibitory to specific strains of the desired species for which the medium was designed.

Appropriate references should be consulted for further information.[14,15]

AVAILABILITY

Cat No.	Description
212269	V-C-A Inhibitor, Ten vials, Lyophilized; each restores to 10 mL
212404	V-C-A-T Inhibitor, Ten vials, Lyophilized; each restores to 10 mL

REFERENCES

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15. Lennette, E.H., A. Balows, W.J. Hausler, Jr., and H.J. Shadomy (ed.), 1985. Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.

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