INSTRUCTIONS FOR USE

VCN ANTIMICROBIC SUPPLEMENT

Freeze-dried selective supplement

1 – INTENDED USE

In vitro diagnostic. Mixture of antimicrobials which is incorporated into culture media to permit the selective isolation of *Neisseria gonorrhoeae* from clinical specimens.

2 – COMPOSITION, TYPICAL FORMULA*

VIAL CONTENTS FOR 500 mL OF MEDIUM

Vancomycin	1.50 mg
Colistin	3.75 mg
Nystatin	6250 UI

* The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

In 1964 Thayer and Martin[1] formulated a selective medium for the cultivation of *Neisseria gonorrhoeae* and *Neisseria meningitidis*, incorporating haemoglobin, yeast supplement B, polymyxin B and ristocetin into GC Agar. Thayer and Martin improved in 1966[2] the formulation substituting the original antibiotics with vancomycin, colistin and nystatin (VCN).
VCN Antimicrobic Supplement is a freeze-dried mixture of antimicrobials to be used as a supplement of GC medium Base for the selective isolation of *Neisseria gonorrhoae*.[3]
Vancomycin inhibits Gram-positive contaminants, colistin inhibits Gram-negative bacteria, including *Pseudomonas* species and almost all saprophytic *Neisseria* spp, nystatin is an anti-fungal agent.

4- DIRECTIONS

Aseptically reconstitute the contents of one vial with 5 mL of sterile purified water and mix gently to dissolve. Use the reconstituted supplement for preparation of Thayer-Martin Medium as described below.
Suspend 19 g of GC Medium Base (REF 401520) in 500 mL of cold purified water; bring to boil stirring constantly and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and aseptically add 5% of defibrinated sheep blood and heat in a water bath at 80°C for 15 minutes. Cool to 47-50°C and add:

- the contents of one vial of Biovitex reconstituted with 5 mL of Restoring Fluid (ref. n° 4240009).
- the contents of one vial of VCN Antimicrobic Supplement reconstituted as described above.

Instead of heated sheep blood, GC medium Base can be supplemented with sterile bovine haemoglobin solution: 5 g of haemoglobin (REF 4122712) in 250 mL of water sterilized by autoclaving + 250 mL of autoclaved GC Medium Base at double concentration. Mix well and distribute into sterile plates.
Final pH of complete medium: 7.2 ± 0.2.

5 – PHYSICAL CHARACTERISTICS

Appearance of lyophilised product	short, dense, yellow pastille
Appearance of reconstituted product	yellow, turbid solution

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Packaging
VCN Antimicrobic Supplement	Supplement for culture media	4240007	10 vials, each for 500 mL of complete medium. Secondary packaging: cardboard box

7 - MATERIALS REQUIRED BUT NOT PROVIDED

GC Medium Base (REF 401520) or other suitable medium, haemoglobin or animal blood, enrichment supplements Biovitex (ref 4240007) autoclave, water bath, incubator and other laboratory equipment. Flasks, sterile plates and tubes, loops and sterile swabs for microbiology, materials for the generation of a controlled incubation atmosphere with CO_2 or CO_2 incubator with humidifier, accessory culture media and reagents for the identification of colonies.

8 – SPECIMENS

Plates of Thayer-Martin Medium prepared as described above, can be directly inoculated with specimens from non-sterile human sites contaminated by mixed flora of bacteria and/or fungi (e.g., urogenital tract, upper respiratory tract, pus and exudates).[4-6] This medium is not useful for the isolation of *Neisseria* spp. from supposedly sterile sites.[7]
Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information because *Neisseria* spp. are very sensitive to collection and storage procedures.[4]

9 – TEST PROCEDURE

Allow plates to come to room temperature. The agar surface should be smooth and moist, but without excessive water.
Process the specimen as soon as possible after it is received in the laboratory to avoid loss of gonococci viability and overgrowth of contaminants.
Roll the swab over one quadrant of the surface then streak the specimen over the other quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap.
Alternatively, since swabs for gonococcal culture may contain only small numbers of organisms, roll the swabs directly on the medium in a large "Z" pattern to sufficiently transfer the specimen; cross-streak the "Z" pattern with a sterile loop.
Incubate at 35-36.5°C in a moist atmosphere supplemented with 3-7% CO_2; cultures should be examined daily for growth and held for a maximum of 72 hours.

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9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
N.gonorrhoeae colonies are variable in size, usually small (0,5-2 mm), moderately convex, raised, granular, glistening, moist, with entire to lobate margins, usually greyish-white to translucent; almost all strains become mucoid after 48 hours.
A Gram staining must be performed on suspected *Neisseria* colonies to confirm the presence of uniform Gram-negative diplococci. Performance of oxidase test is mandatory for colonies suspected to belong to *Neisseria* that shall be positive for *N.gonorrhoeae*.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[8]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
Thayer-Martin Medium				
N.gonorrhoeae	ATCC	43069	35-36,5°C / 24-48H / CO_2	good growth
P.mirabilis	ATCC	43071	35-36,5°C / 24-48H / CO_2	inhibited
E.coli	ATCC	25922	35-36,5°C / 24-48H / CO_2	inhibited
N.sicca	ATCC	9913	35-36,5°C / 24-48H / CO_2	growth partially inhibited
S.epidermidis	ATCC	12228	35-36,5°C / 24-48H / CO_2	inhibited
C.albicans	ATCC	60193	35-36,5°C / 24-48H / CO_2	growth partially inhibited

ATCC is a trademark of American Type Culture Collection

12- PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of VCN Antimicrobic Supplement are / is?? tested for productivity and selectivity properties with Thayer-Martin Medium plates.
Productivity is tested by semi-quantitative ecometric technique with 2 gonococcal strains: *N.gonorrhoeae* ATCC 43069*, N.gonorrhoeae* ATCC 19424. After incubation at 35-36.5°C for 24-48 hours, with 3-7% of CO_2, the amount of growth is evaluated and recorded. All strains show a good growth with typical morphology. The selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target organisms *N.sicca* ATCC 9913, *S.epidermidis* ATCC 12228*, E.coli* ATCC 25922*, P.mirabilis* ATCC 43071*, P.rettgeri ATCC 39944, S.aureus ATCC 25923, E.faecalis ATCC 19433 and C.albicans* ATCC 60193. After incubation at 35-36.5°C for 24-48 hours, with 3-7% of CO_2, the growth of *Proteus* spp. is partially inhibited while the growth of other non-target strains is inhibited.

13 - LIMITATIONS OF THE METHOD

- Vancomycin sensitive strains of some auxotypes of *N.gonorrhoeae* which fail to grow on Thayer-Martin Medium, have been reported from 3% to 10% of the total isolates.[9,10]
- It is recommended that both a selective and a non-selective medium be used when isolating pathogenic *Neisseria* in order to avoid the loss of vancomycin sensitive strains.[7]
- Thayer-Martin Medium is not useful for the isolation of *Neisseria* spp. from supposedly sterile sites as cerebrospinal fluid, conjunctival swab, skin biopsy, joint fluid for which non-selective media are recommended.[7]
- For the growth of *N.gonorrhoeae* it is necessary that the surface of the plates is moist; if it appears dry, humidify with a few drops of sterile distilled water. Place damp gauze or paper towels in the CO_2 container before incubation or use an incubator with humidifier.[7]
- On Thayer-Martin Medium *N.gonorrhoeae* grows with smaller and more granular colonies than with non-selective chocolate agar.
- Some saprophytic non-target microorganisms, resistant to antimicrobials present in the media may grow. *N.lactamica* may grow on TM with colonies smaller and less moist than gonococci, occasionally with a yellowish tint.[7]
- The gonococci are one of the most fragile Gram-negative bacteria. It is recommended that any suspected *Neisseria* containing specimen should be inoculated onto primary isolation medium immediately on collection to avoid any loss in viability and/or overgrowth of contaminants; if this is not possible *N.gonorrhoeae* swabs are better held at 4-6° C for not more than 3 hours.[7]
- The incubator temperature should be set at 35-36,5°C[11] because many strains of *N.gonorrhoeae* will not grow well at 37°C. [7,12]
- Examine plates after 24 hours incubation. At 48 hours the Gram morphology may exhibit atypical forms.
- Many standard protocols[13-15] describe the use of Thayer-Martin medium for the detection of meningococcal carriage in oropharyngeal and nasopharyngeal swabs. This application is out the intended use of GC Medium base supplemented with Biovitex and VCN supplements The end user should validate this application before routinely using those selective media for *N.meningitidis* detection in clinical specimens.
- Use dacron or calcium alginate swabs for specimen collection, avoid cotton swabs since they contain fatty acids which are inhibitory for *N.gonorrhoeae*.[7]
- Incorrect specimen collection, incubation temperature, CO_2 level, humidity and pH can adversely affect growth and viability of the microorganisms.
- Inactivation or deterioration of antibiotics into selective media can allow the growth of contaminants.
- It is recommended to measure the pH of complete media. GC Medium Base has sufficient buffering capability however sometimes it could be necessary to adjust the final pH.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- The VCN Antimicrobic Supplement and the prepared media are intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- VCN Antimicrobic Supplement is a qualitative *in vitro* diagnostic, for professional use only; it must be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.

- VCN Antimicrobic Supplement is classified as dangerous according to current European legislation; consult the Safety Data Sheet before use.
- The supplement and the medium base shall be used in association according to the directions described above. Apply Good Manufacturing Practice in the preparation process of plated media.
- VCN Antimicrobic Supplement is sterilized by membrane filtration.
- Be careful when opening the metal ring to avoid injury.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplements or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused supplements and the sterilized media inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use VCN Antimicrobic Supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets of the products are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store the product in the original package at 2-8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).
The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes) and the applied storage conditions (temperature and packaging).

16 – REFERENCES

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15. Public Health England: Standards for microbiology investigations (UK SMI)- Bacteriology: UK SMI B2:2017, UK SMI B9:2015, UK SMI B14:2016; UK SMI B28:2017; B51:2014

VCN ANTIMICROBIC SUPPLEMENT REF 4240007
SDS rev 6
Regulation (EU) 2020/878

Classification
The product is classified as hazardous. The product thus requires a safety data sheet that complies with the provisions of (EU) Regulation 2020/878.
Hazard classification and indication:

Acute toxicity, category 4	H302	Harmful if swallowed.
Skin sensitization, category 1	H317	May cause an allergic skin reaction.

Labelling
Hazard pictograms:

Signal words: Warning
Hazard statements:
H302 Harmful if swallowed.

H317 May cause an allergic skin reaction.
Precautionary statements:
P280 Wear protective gloves.
P261 Avoid breathing dust / fume / gas / mist / vapours / spray.
P333+P313 If skin irritation or rash occurs: Get medical advice / attention.
P264 Wash . . . thoroughly after handling.
P362+P364 Take off contaminated clothing and wash it before reuse.
Contains: VANCOMYCIN HCL, COLISTIN

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	This side up	
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2021/12
Revision 2	Removal of obsolete classification	2023/04

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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