INSTRUCTIONS FOR USE

BIOVITEX-RESTORING FLUID

Freeze-dried enrichment supplement

1 – INTENDED USE

In vitro diagnostic. Chemically defined supplement used as an additive to culture media for cultivation of nutritionally fastidious microorganisms

2 - COMPOSITION-TYPICAL FORMULA *

BIOVITEX: VIAL CONTENT

		Vial for 500 ml of medium (5mL) REF 4240009	Vial for 5 L ml of medium (50 mL) REF 42185011	Equivalent concentration per litre of medium
Vitamin B_{12}	mg	0.05	0.5	0.1
L-Glutamine	mg	50	500	100
Adenine	mg	5	50	10
Guanine HCl	mg	0.15	1.5	0.3
p-Aminobenzoic acid	mg	0.065	0.65	0.13
L-Cystine	mg	5.5	55	11
NAD (Coenzyme 1)	mg	1.25	12.5	2.5
Cocarboxylase	mg	0.5	5	1
Ferric nitrate	mg	0.1	1	0.2
Thiamine HCl	mg	0.015	0.15	0.03
L-Cysteine HCl	mg	129.5	1295	259

RESTORING FLUID: VIAL CONTENT

		Vial for 500 ml of medium (5mL) REF 4240009	Vial for 5 L ml of medium (50 mL) REF 42185011	Equivalent concentration per litre of medium
Glucose	g	0.5	5	1
Purified water	ml	5	50	10

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Biovitex is a freeze-dried mixture of growth factors to be used as a supplement for the cultivation of microorganisms with specific nutritional requirements (*Neisseria, Haemophilus,* etc.). Biovitex can be used for the preparation of chocolate agar enriched[1], Thayer-Martin medium[1,2] and other culture media for the cultivation of fastidious microorganisms[3].
Biovitex provides factor V (NAD) for the growth of *Haemophilus* spp., vitamins, amino acids, coenzymes, glucose, iron and other factors that enhance the growth of Neisseria spp.[4]

4A- DIRECTIONS FOR RECONSTITUTION

Each vial of Biovitex is supplied with a vial of Restoring Fluid.
Dissolve the Biovitex lyophilized product with the contents of a vial of Restoring Fluid, under aseptic conditions. The volume obtained is 5 mL for code 4240009 and 50 mL for code 42185011.
Generally, 5 mL of Biovitex (REF 424009) are added to 500 mL of GC medium base, 50 mL of Biovitex (REF 42185011) are added to 5 litres of GC medium base.

4B- DIRECTIONS FOR MEDIA PREPARATION

Chocolate agar enriched (haemoglobin)
Prepare a double strength GC Medium Base by suspending 38 g of in 500 mL of purified water. Mix thoroughly, heat with frequent agitation and boil for about 1 min.
Suspend 10 g haemoglobin powder in 500 mL purified water to make a 2% solution.
Autoclave separately the GC Medium Base and haemoglobin solution at 121 °C for 15 min.
Cool the autoclaved solutions to approximately 47-50 °C.
Reconstitute Biovitex enrichment as described above.
Aseptically add 500 mL of haemoglobin solution and 10 mL of Biovitex to the 500 mL of GC Medium Base.
Mix gently but thoroughly and distribute into sterile Petri dishes or tubes, or other sterile containers.
final pH 7.2 ± 0.2
Chocolate agar enriched (cooked blood)
To 500 mL of sterilized GC Medium Base, cooled to 47-50°C, aseptically add 5-10% of the defibrinated horse blood and heat to 80°C for 15 minutes. Cool to 47-50°C and carefully add 5 mL of Biovitex reconstituted as described above. Mix well and distribute and distribute into sterile Petri dishes or tubes or other sterile containers. final pH 7.2 ± 0.2
Selective media for Neisseria (TM and MTM)
To 500 mL of sterilized GC Medium Base, cooled to 47-50°C, aseptically add 5% of the defibrinated sheep blood and heat to 80°C for 15 minutes. Cool to 47-50 ° C and add 5 mL of reconstituted Biovitex as described above. In addition, add the contents of one vial of VCN Antimicrobic Supplement (cat. No. 4240007), reconstituted with 5 mL of sterile purified water (Thayer-Martin medium). Alternatively, add the contents of one vial of VCNT Antimicrobic Supplement (cat. No. 4240008) reconstituted with 5 mL of sterile purified water (modified

Thayer-Martin's medium). Instead of heated sheep blood, GC Medium Base can be supplemented with a sterile solution of bovine hemoglobin: 10 g of hemoglobin in 500 mL of water, sterilized in an autoclave + 500 mL of double strength GC Medium Base, autoclaved. Mix well and distribute onto sterile Petri dishes. final pH 7.2 ± 0.2

5 – PHYSICAL CHARACTERISTICS

Appearance of lyophilised Biovitex	short, close, pink pastille
Appearance of Restoring Fluid	colourless, limpid solution
Appearance of reconstituted Biovitex	pale pink, limpid solution

6 - MATERIALS PROVIDED - PACKAGING

Product	Tipo	REF	Confezione
Biovitex-Restoring Fluid	Supplement for culture media	4240009	5 vials of Biovitex+5 vials of 5 mL Restoring Fluid for 500 mL of complete medium. Secondary packaging: cardboard box
Biovitex-Restoring Fluid	Supplement for culture media	42185011	1 vial of Biovitex+1 vial of 50 mL Restoring Fluid for 5000 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, selective supplements VCN (ref 4240007) or VCNT (REF 4240008), 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

The clinical samples to be tested with culture media prepared with Biovitex depend on the medium used and the purpose of the analysis. Refer to the literature cited for the selection of the most appropriate specimen according to the specific infection.[4-9] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied.[9]

9 – TEST PROCEDURE

Allow plates to come to room temperature. 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.
The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols. Consult the references for further information[6-9] or the GC Medium Base Instructions for Use (REF 401520).

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic characteristics of the colonies.
The characteristics of the colonies depend on the culture medium used and the microorganisms isolated. For further information consult the literature references[6-9] or the GC Medium Base Instructions for Use (REF 401520).

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.

TEST STRAINS			INCUBATION (T°/ t / ATM)	EXPECTED RESULTS
Chocolate agar enriched				
H.influenzae	ATCC	10221	35-37°C / 18-24H / CO_2	good growth
N.gonorrhoaes	ATCC	43069	35-37°C / 18-24H / CO_2	good growth
Thayer-Martin and Modified Thayer-Martin Media				
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
Haemophilus selective medium				
H.influenzae	ATCC	10221	35-37°C / 24-48H / CO_2	good growth
S.pyogenes	ATCC	19615	35-37°C / 44-48H / CO_2	growth 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 Biovitex-Restoring Fluid are tested for productivity with chocolate agar enriched plates by semi-quantitative ecometric technique with the following strains: *H.influenzae* ATCC 10221, *H.influenzae* ATCC 19418, *N.gonorrhoeae* ATCC 43069, *N.gonorrhoeae* ATCC 19424, *S.pneumoniae* ATCC 6301, *S.pyogenes* ATCC 12834, *S.aureus* ATCC 25923. After incubation at 35-37°C for 18-24 hours, in aerobic conditions or with 5-10% of CO_2, all the tested strains show good growth.

13 - LIMITATIONS OF THE METHOD

Biovitex and GC Medium Base used for the preparation of chocolate agar enriched

- The growth on chocolate agar enriched depends on the metabolic requirements of each microorganism; it is possible that some strains are unable to grow on the medium.
- Depending on the specimens analysed and the microorganisms being tested for, it is recommended to use also additional selective media such as Thayer-Martin for the isolation of gonococcus and Haemophilus selective agar for the isolation of *H.influenzae*.
- 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.[5]
- 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.[5]
- If *N.gonorrhoeae* is suspected the incubator temperature should be set at 35-36.5°C with 5% CO_2, because many strains of *N. gonorrhoea*e will not grow well at 37°C and grow poorly with 10% CO_2.[5,11]
- The number and type of fastidious species present in the specimens as infectious agents is very high. Therefore, before the chocolate agar enriched is routinely used for rarely isolated or recently described microorganisms, its suitability must be verified by the user.
- The presence of colonies on chocolate agar enriched is not an indication, by itself, of the presence of pathogenic microorganisms: user must differentiate potential pathogens requiring biochemical, immunological, molecular, or mass spectrometry testing for identification and antimicrobial testing from contaminants that represent member of normal microbiota.

Biovitex and GC Medium Base used for the preparation of Thayer-Martin and Modified Thayer-Martin media

- Vancomycin sensitive strains of some auxotypes of *N.gonorrhoeae* which fail to grow on MTM, have been reported from 3% to 10% of the total isolates.[12,13] Some gonococci are susceptible to trimethoprim too.[14]
- 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 and/or trimethoprim sensitive strains.[5]
- TM and MTM are 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.[5]
- 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.[5]
- On TM and MTM *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 and MTM with colonies smaller and less moist than gonococci, occasionally with a yellowish tint.[5]
- 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.[5]
- The incubator temperature should be set at 35-36,5°C[11] because many strains of *N.gonorrhoea*e will not grow well at 37°C. [5,11]
- Examine plates after 24 hours incubation. At 48 hours the Gram morphology may exhibit atypical forms.
- Many standard protocols[4,7,8,9] describe the use of Thayer-Martin and Modified Thayer-Martin media for the detection of meningococcal carriage in oropharyngeal and nasopharyngeal swabs. This application is out the intended use of of GC Medium base supplemented with Biovitex and selective supplements. The end user should validate this application before routinely using those selective media for *N.meningitidis* detection in clinical specimens.

All media prepared with Biovitex and GC Medium Base

- Use dacron or calcium alginate swabs for specimen collection, avoid cotton swabs since they contain fatty acids which are inhibitory for *N.gonorrhoeae*.[5]
- 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 Biovitex 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

- Biovitex-Restoring Fluid 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.
- Biovitex 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 or tubed media.
- The Biovitex supplement is sterilized by membrane filtration, while the Restoring Fluid is subjected to autoclaving.
- 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 the Biovitex-Restoring Fluid 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

1. CDC: Lab Manual, meningitidis; Annex: Preparation of Media and Reagents, 2016
2. Martin JE Jr, Billings TE, Hackney JF, Thayer JD. Primary isolation of N.gonorrooeae with a new commercial medium. Public Health Rep. 1967; 82:361-363.
3. Gonzales MD, Ledeboer NA. Haemophilus. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
5. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.270.
6. Vandepitte J, Verhaegen J, P. Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd edition Geneve: World Health Organization Geneva; 2003.
7. 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
8. Elias J, Frosh M, Vogel U. Neisseria. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.635.
9. Ledeboer NA, Doern GV. Haemophilus. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.667.
10. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019.
11. CDC: Morbidity and Mortality Weekly Report (MMWR). Screening Tests To Detect Chlamydia trachomatis and Neisseria gonorrhoeae. Infections Recommendations and Reports. October 18, 2002 / Vol. 51 / No. RR-15
12. Talbot V. et al. Vancomycin sensitive penicillinase producing Neisseria gonorrhoea. Br.J Ven Dis. 1983; 59:277
13. Mirret S, Reller B, Knapp JS. Neisseria gonorrhoeae Strains inhibited by vancomycin in selective media and correlation with auxotype.J Clin Microbiol 1981; 14: 94
14. Lai-King Ng, Martin IE.The laboratory diagnosis of Neisseria gonorrhoeae Can J Infect Dis Med Microbiol. 2005; 16(1): 15–25.

4240009 / 42185011 **BIOVITEX**
SDS
Regulation (EU) 2020/878

Contains:
L-CYSTEINE HCL

Classification

Eye irritation, category 2	H319	Causes serious eye irritation.
Skin irritation, category 2	H315	Causes skin irritation.
Specific target organ toxicity - single exposure, category 3	H335	May cause respiratory irritation.

Labelling
Hazard pictograms:

Signal words: Danger
Hazard statements:
H319 Causes serious eye irritation.
H315 Causes skin irritation.
H335 May cause respiratory irritation.
Precautionary statements:
P261 Avoid breathing dust / fume / gas / mist / vapours / spray.
P280 Wear protective gloves / eye protection / face protection.
P312 Call a POISON CENTRE / doctor / . . . if you feel unwell.
P403+P233 Store in a well-ventilated place. Keep container tightly closed.
P264 Wash . . . thoroughly after handling.

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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