INSTRUCTIONS FOR USE

GARNERELLA ANTIMICROBIC SUPPLEMENT

Freeze-dried selective supplement

1 - INTENDED USE

In vitro diagnostic. Mixture of antimicrobials to be added to Columbia Agar Base for the isolation of *Gardnerella vaginalis* from clinical specimens.

2 - COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Gentamicin sulphate	2 mg
Nalidixic acid sodium salt	15 mg
Amphotericin B	1 mg

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

G.vaginalis is a facultative anaerobic, non-motile, pleomorphic, Gram-negative or Gram-variable bacillus, oxidase and catalase negative.
A normal vaginal flora is characterized by the presence of only *Lactobacillus* species or with the presence of small numbers of *G.vaginalis* morphotypes; the shift in vaginal flora associated with bacterial vaginosis is characterised by a decrease in numbers of lactobacilli which are replaced by a mixed flora of aerobic, anaerobic and microaerophilic species, including *G.vaginalis*.[1]
Diagnosis of bacterial vaginosis is based on the Amsel criteria, which is considered 90% accurate with three or four of the following findings: 1-thin, white, yellow homogeneous vaginal discharge, 2-amine (fishy) odour when potassium hydroxide solution is added to vaginal secretions (commonly called the "whiff test"), 3-presence of clue cells (greater than 20%) on microscopy, 4-vaginal pH greater than 4.5.[2] The isolation of *G.vaginalis* from vaginal swab can support the diagnosis of bacterial vaginosis.[3]
Gardnerella Antimicrobic Supplement is based on the use of selective compounds proposed by Ison et al.[4] Gentamicin is effective against both Gram-positive and Gram-negative organisms other than *G vaginalis*[4], nalidixic acid is inhibitory is primarily against Gram-negative bacteria, with minor anti-Gram-positive activity, amphotericin B is an antifungal agent. Gardnerella Antimicrobic Supplement may be used for the preparation of the isolation media using Columbia Agar Base and sheep or horse or rabbit or human blood.[4,5]

4- DIRECTIONS

Reconstitute the contents of one vial with 2 mL of a 1:1 (v/v) mixture of ethanol and sterile purified water. Aseptically add the contents to 500 mL of autoclaved Columbia Agar Base cooled to approximately 47-50°C, and supplemented with 25-50 mL of defibrinated sterile human, rabbit, sheep or horse blood. Mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Freeze-dried supplement appearance	short, dense, yellow pastille
Reconstituted supplement appearance	opalescent, yellow

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Gardnerella Antimicrobic Supplement	Freeze-dried supplement	4240019	10 vials, each for 500 mL of medium

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Columbia Agar Base (REF 401136), human, rabbit, horse or sheep blood, ethanol, autoclave, water-bath, incubator and laboratory equipment as required, sterile loops and swabs, Petri dishes, Erlenmeyer flasks, CO_2 generators and jars or CO_2 incubator with humidifier, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Columbia Agar Base supplemented with defibrinated blood and Gentamicin Antimicrobic Supplement can be directly inoculated with vaginal and extra-vaginal specimens collected with cotton tipped swab. It is best to take one swab for direct examination and to take another for culture.[6] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[6]

9 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate by rolling the swab over a small area of the surface at the edge and streak with a sterile loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap.
Incubate at 35–37°C in 5–10% carbon dioxide for 44-48 hours.

9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
G.vaginalis grows with white, small (<0.5 mm in diameter) colonies.
G. vaginalis is beta-haemolytic on media containing human or rabbit blood but not on sheep or horse blood agar.[5] The addition of Tween 80 (0.02% v/v) to the medium containing human blood improves haemolysis and enhances growth.[5]

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 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.

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CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
G.vaginalis	ATCC 14018	35-37°C / 48H / CO_2	growth
C.albicans	ATCC 18804	35-37°C / 48H / CO_2	inhibited
E.faecalis	ATCC 19433	35-37°C / 48H / CO_2	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 Gardnerella Antimicrobic Supplement added to Columbia Agar Base together with 10% of defibrinated sheep blood, is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity characteristics are tested by semi-quantitative ecometric technique and modified Miles-Misra surface drop method with the target strains *G.vaginalis* ATCC 49145 and ATCC 14018. After incubation at 35-37°C for 48 hours in 5 to 10% CO_2 atmosphere the amount of growth is evaluated and recorded. The strains show a good growth with non-haemolytic colonies. 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 *E.coli* ATCC 25922*, P.mirabilis* ATCC 100053*, E.faecalis* ATCC 19433*, S.aureus* ATCC 25923*, L.acidophilus* ATCC 314*, C.albicans* ATCC 18804. After incubation at 35-37°C for 48 hours, the growth of *P.mirabilis* is partially inhibited while the growth of the other non-target strains is totally inhibited.

13 - LIMITATIONS OF THE METHOD

- The presence of *G. vaginalis* in a vaginal specimen does not necessarily indicate that the isolated organism is the cause of an infection.
- 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 complete culture medium is 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

- The 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.
- The 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 production process of prepared media.
- The 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 the 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 Sheet of the product 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 and the applied storage conditions (temperature and packaging).

16 - REFERENCES

1. Public Health England. Investigation of Genital Tract and Associated Specimens. UK Standards for Microbiology Investigations. 2017, B 28 Issue 4.6.
2. Colonna C, Steelman M. Amsel Criteria. *In*Statpearls. StatPearls Publishing; Last update, July 1, 2019
3. Funke G, Bernard KA. Coryneform Gram-Positive Cocci. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.474
4. Ison CA. Dawson SG, Hilton J, Csonka GW, Easmon CSF. Comparison of culture and microscopy in the diagnosis of Gardnerella vaginalis infection. J. Clin. Pathol 1982; 35:550
5. Catlin BV. Gardnerella vaginalis: characteristics, clinical considerations, and controversies. ClinMicrobiol Rev 1992; 5:213
6. 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.

GARDNERELLA ANTIMICROBIC SUPPLEMENT REF 4240019
SDS rev 5
Regulation (EU) 2020/878

Hazardous ingredient: nalidixic acid sodium salt, gentamicin sulphate, amphotericin B

Hazard classification and indication:

Acute toxicity, category 4	H302	Harmful if swallowed.
Specific target organ toxicity - single exposure, category 3	H335	May cause respiratory irritation.
Skin sensitization, category 1	H317	May cause an allergic skin reaction.

Labelling
Pictogram

Hazard	Warnings
Hazard statements:	
H335	May cause respiratory irritation.
H317	May cause an allergic skin reaction.
Precautionary Statements	
P280	Wear protective gloves.
P261 A	void breathing dust / fume / gas / mist / vapours / spray.
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.
P362+P364	Take off contaminated clothing and wash it before reuse.

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	2022/11
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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