INSTRUCTIONS FOR USE

GARDNERELLA SELECTIVE AGAR

Ready-to-use plates

Gardnerella Selective Agar:
Gardnerella vaginalis

1 - INTENDED USE

In vitro diagnostic device. Selective medium with defibrinated sheep blood for the isolation of *Gardnerella vaginalis* from vaginal discharge.

2 - COMPOSITION - TYPICAL FORMULA *

Peptocomplex	10 g
Tryptose	10 g
Peptone	3 g
Maize Starch	1 g
Sodium Chloride	5 g
Agar	12 g
Defibrinated sheep blood	50 mL
Gentamicin sulphate	4 mg
Nalidixic acid	30 mg
Amphotericin B	2 mg
Purified water	1000 mL

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

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]
Although not recommended for routine laboratory procedures, the isolation of *G.vaginalis* can support the diagnosis of bacterial vaginosis.[3]
Gardnerella Selective Agar is based on the use of selective compounds proposed by Ison[4] and the observation that the colony sizes on Columbia agar base with 5% sheep blood are optimal[5].
Peptones provide carbon, nitrogen and trace elements for bacterial growth, sodium chloride maintains the osmotic balance, maize starch is included to absorb toxic by-products contained in the specimen, sheep blood enhances the growth of *G.vaginalis,* though it is not useful for the differentiation of *G.vaginalis* colonies that are β-haemolytic only with human and rabbit blood. Gentamicin is inhibitory for Gram-negative and Gram-positive organisms other than *G vaginalis*[4], nalidixic acid is inhibitory for Gram-negative bacteria, amphotericin B is an antifungal agent.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	red, opaque
Final pH at 20-25°C	7.3 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Gardnerella Selective Agar	Ready-to-use plates	549993	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, CO_2 generators and jars or CO_2 incubator, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Gardnerella Selective Agar can be directly inoculated with vaginal swab. It is best to take one swab for direct examination and to take another for culture.[3] Good laboratory practices for collection, transport and storage of the clinical specimens should be applied.[6]

8- 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.

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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.
G.vaginalis colonies are white, small (<0,5 mm in diameter), non β-haemolytic.

10 - 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, it is responsibility of the end-user to perform 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.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
G.vaginalis	ATCC	14018	35-37°C / 44-48H / CO_2	good growth, non β- haemolytic colonies
C.albicans	ATCC	10231	35-37°C / 44-48H / CO_2	inhibited
E.faecalis	ATCC	29212	35-37°C / 44-48H / CO_2	inhibited

ATCC is a trademark of American Type Culture Collection

11- PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of ready-to-use plates of Gardnerella Selective Agar and of the raw materials used for the production of prepared plates (dehydrated Columbia Agar Base REF 401136, supplemented with sheep blood and Gardnerella Selective Supplement REF 4240018) are tested for productivity and selectivity by incubating at 35-37°C for 44-48°C in 5-10% CO_2, comparing the results with a previously approved Reference Batch.
Productivity is tested by semi-quantitative ecometric technique with the target-strain *G.vaginalis*ATCC 14018. The amount of growth on the plates after incubation is evaluated and shall be comparable in both batches.
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 strains *E.faecalis* ATCC 29212, *S.aureus* ATCC 25923, *E.coli* ATCC 25922, *P.mirabilis* ATCC 10005, *L.acidophilus* clinical isolate, *C.albicans* ATCC 10231. After incubation at 35-37°C for 44-48°C in 5-10% CO_2, the growth of non-target strains *S.aureus*, *E.coli*, *L.acidophilus*, is totally inhibited while the growth of *E.faecalis P.mirabilis C.albicans* is partially inhibited.

12 - LIMITATIONS OF THE METHOD

- Since the medium doesn't contain human or rabbit blood, the presumptive identification of *G.vaginalis* colonies by β-haemolysis evidence is not possible.
- 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.
- This 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.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that these products do not contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use plates be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- 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.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - 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.

TABLE OF APPLICABLE SYMBOLS

REF or **REF** Cataloguenumber	**LOT** Batch code	**IVD** *In vitro*Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contentssufficient for <n>tests	ConsultInstructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2020/11
Revision 2	Removal of obsolete classification	2023/03

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

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