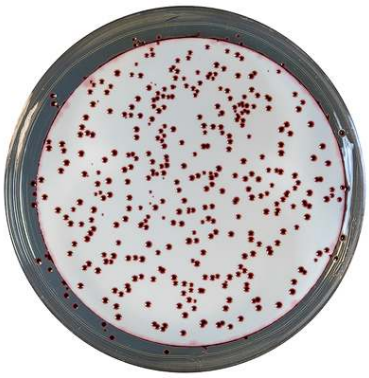
SLANETZ BATLEY AGAR

Dehydrated and ready-to-use culture medium



Slanetz Bartley Agar: colonies of *Enterococcus faecalis*

1 - INTENDED USE

Selective and differential medium for the enumeration of enterococci in water and in other materials by the membrane filtration technique.

2 - COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED AND READY-TO USE MEDIUM

Tryptose	20.00 g
Yeast extract	5.00 g
Glucose	2.00 g
Potassium phosphate bibasic	4.00 g
Sodium azide	0.40 g
Triphenyltetrazolium chloride (TTC)	0.10 g
Agar	10.00 g

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Slanetz Bartley Agar is a selective and differential medium prepared according to the formulation devised by Slanetz, Bent and Bartley[1] and later modified by Slanetz and Bartley[2] with the introduction of triphenyltetrazolium chloride (TTC). Slanetz Bartley Agar, also called mEnteroccous agar or m-Azide Agar, meets the requirements of ISO 7899-2[3] and APHA[4] for the enumeration of intestinal enterococci in water using the membrane filtration technique. Burkwall and Hartman[5] demonstrated that the addition of 0.5 mL of Tween 80 and 20 mL of a 10% sodium carbonate or bicarbonate solution to each litre of the medium was valuable when investigating enterococci in frozen foods. The method described in ISO 7899-2 involves enumeration of intestinal enterococci with membrane filters on Slanetz Bartley Agar medium, followed by confirmation on Bile Aesculin Azide Agar.
Tryptose and yeast extract provide nitrogen, carbon, vitamins, amino acids and trace elements for microbial growth. Glucose is a source of carbon and energy, dipotassium phosphate buffers the medium and sodium azide is the selective agent to suppress the growth of Gram-negative bacteria. TTC acts as an indicator: enterococci reduce it to insoluble formazan inside the bacterial cells and grow with red/brown/pink colonies.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 41.5 g of in 1000 mL of cold purified water. Heat to boiling with frequent agitation. Do not exceed heating time and temperature, do not autoclave. Cool to approximately 47-50 °C, mix well and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Prepared medium appearance	yellow with pink hues, clear or slightly opalescent
Final pH at 20-25 °C	7.2 ± 0.1

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Slanetz Bartley Agar	Dehydrated medium	4020462 4020464	500 g (12.1 L) 5 kg (121 L)
Slanetz Bartley Agar	Ready-to-use plates	542046	2 x 10 plates ø 90 mm
Slanetz Bartley Agar	Ready-to-use plates	492046	3 x 10 plates ø 55 mm

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, membrane filters, ancillary culture media and reagents.

8 – SPECIMENS

The method of analysis described here, taken from ISO 7899-2, is suitable for the examination of drinking water, water from swimming pools and other clean or disinfected water. However, the method can be applied to all types of water with the exception of water with a high amount of suspended matter or considerable load of interfering microorganisms. The application of the method appears particularly appropriate for the analysis of large quantities of water containing low number of intestinal enterococci. Refer to the cited Standard[3,4] and other applicable Standards for operational sampling details.

9 - TEST PROCEDURE,

Membrane filter technique[3]

1. Filter a suitable volume of sample through a 0.45 µm membrane filter.
2. Place the membrane on a Slanetz Bartey Agar plate and incubate at 36 ± 2°C for 44 ± 4 hours.
3. After incubation, consider as typical all colonies showing red, brown or pink colour.
4. If typical colonies are observed, transfer the membrane to the surface of a Bile Aesculin Azide Agar ISO Formulation plate (REF 401018) and incubate at 44 ± 0.5°C for 2 hours.

10- READING AND INTERPRETATION

After incubation, observe the bacterial growth, recording each specific morphological and colour characteristic of the colonies.
Count as intestinal enterococci all colonies red-brown or pink on Slanetz Bartley Agar and which grow with a brown to black halo on Bile Aesculin Azide Agar.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

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.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. faecalis ATCC 29212	35-37°C /44-48 H-A	good growth, red colonies
E. faecium ATCC 6057	35-37°C /44-48 H-A	good growth, red colonies
E. coli ATCC 25922	35-37°C /44-48 H-A	totally inhibited
S. aureus ATCC 25923	35-37°C /44-48 H-A	totally inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, representative samples of all lots of dehydrated and ready-to-use Slanetz Bartley Agar (TB) are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch and Tryptic Soy Agar (TSA).
Productivity is tested by a quantitative MF method with the following target strains *E. faecalis* ATCC 29212, *E. faecalis* ATCC 19433, *E. faecalis* CIP 106877, *E. faecium* ATCC 6057, *E. faecium* CIP 106876. The membrane filters on Slanetz Bartley Agar plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 37°C for 44 hours. The colonies are enumerated on tested batches and the productivity ratio (Pr: CFU_{TB}/CFU_{TSA}) is calculated. If Pr is ≥ 0.5 and if the colonies morphology and colour are typical (red colonies) the results are considered acceptable and conform to the specifications
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. coli* ATCC 25922 and *S. aureus* ATCC 25923. After incubation for 44* hours at 37°C, the growth of non-target strains is totally inhibited.

13 – LIMITATIONS OF THE METHOD

- ISO 7899-2 describes a method for the isolation and enumeration of intestinal enterococci, mainly belonging to the species *E. faecalis*, *E. faecium*, *E. durans* and *E. hirae*. In addition, other species referable to the genus *Enterococcus* and some species referable to the genus *Streptococcus* (i.e., *S. bovis* and *S. equinus*) may occasionally be detected. These *Streptococcus* species do not survive long in water and it is likely that a quantitative assessment is not possible. For the purposes of water testing, enterococci may be considered as indicators of faecal pollution. However, it should be noted that some enterococci found in water may also occasionally originate from different habitats.[3]
- In the confirmation test performed with filter membrane transfer, an uneven distribution of bacterial colonies or the presence of high microbial loads may interfere with the differentiation of positive colonies due to the spread of colour to adjacent colonies.

14 - PRECAUTIONS AND WARNINGS

- This culture medium is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Slanetz Bartely Agar is classified as hazardous. Before use, consult the Safety Data Sheet.
- 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 this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium 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.
- Apply Good Manufacturing Practice in the production process of prepared media.
- 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheets of the product 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.

15 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
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). According to ISO 7889-2, prepared plates can be stored in the dark and protected against evaporation for up to 2 weeks at 5 °C ± 3 °C.

Ready to use plates

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, pronounced pink colour, excess of moisture).

16 – REFERENCES

1. Slanetz LW, Bent DF, Bartley CH. Use of the membrane filter technique to enumerate enterococci in water. Public Health Rep (1896),1955;70:67-72.
2. Slanetz LW, Bartley CH. Numbers of enterococci in water, sewage, and feces determined by the membrane filter technique with an improved medium. J Bacteriol 1957; 74:591-5.
3. ISO 7899-2:2000 Water quality — Detection and enumeration of intestinal enterococci — Part 2: Membrane filtration method.
4. APHA Standard Methods for the Examination of Water and Wastewater. 23th ed. American Public Health Association, Washington, D.C. 2017.
5. Burkwall MK, Hartman PA. Comparison of direct plating media for the isolation and enumeration of enterococci in certain frozen foods. Appl Microbiol. 1964; 12:18-23.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 4	Updated layout and content	2022/06

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

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it