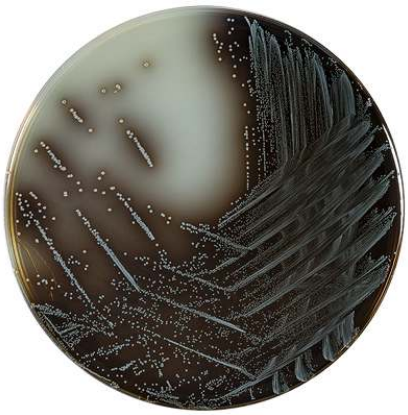
BILE AESCULIN AGAR

Dehydrated culture medium



Bile Aesculin Agar: colonies of *Enterococcus faecalis*

1 - INTENDED USE

Differential medium for the confirmation test of enterococci colonies isolated from food and water.

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

Beef extract	3.0 g
Peptone	5.0 g
Oxgall	40.0 g
Ferric citrate	0.5 g
Aesculin	1.0 g
Agar	14.0 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

In 1924, Rochaix[1] applied aesculin test for the differentiation of enterococci from other streptococci. Utilizing the esculin base, Meyer and Schonfeld[2], in 1926 then added bile: in their study they found that enterococci were able to grow and split esculin, whereas other streptococci could not. Swan[3] in his study used the esculin medium containing 4% bile salt.
Bile Aesculin Agar, prepared according to the formulation of Swan[3] and recommended by APHA[4] and MSDA[5], is used for the confirmation test of enterococci colonies isolated from water or food samples based on their ability to grow and hydrolyse the glycoside esculin in the presence of bile.[6] The use of this medium in conjunction with triple sugar iron agar and lysine iron agar has been proposed for differentiating the *Klebsiella-Enterobacter-Serratia* group from other *Enterobacteriaceae*.[7]
Peptone and beef extract provide nitrogen, carbon, amino acids and trace elements for microbial growth; bile salts are selective agents that limit the growth of Gram-positive bacteria other than enterococci; esculin is hydrolysed by this group of bacteria to glucose and aesculetin (6-7dihydroxycoumarin): aesculetin reacts with ferric citrate to form a dark brown or black complex.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 64 g in 1000 ml of cold purified water. Heat to boiling with frequent agitation, cool to 47-50°C and distribute into sterile Petri dishes. Do not overheat.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared medium appearance	dark amber, slight opalescent
Final pH at 20-25 °C	6.4 ± 0.2

6 - MATERIALS PROVIDED

Product	Type	REF	Pack
Bile Aesculin Agar	Dehydrated medium	4010172	500 g (7.8 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes and tubes, ancillary culture media and reagents for the complete identification of the isolated strains.

8 – SPECIMENS

The sample consists of bacterial colonies isolated from food or water samples by the usual techniques, grown on selective media for enterococci.

9 - TEST PROCEDURE, READING AND INTERPRETATION

Pick suspect colonies grown on enterococcal selective medium and perform a four-quadrant streak onto a Bile Aesculin Agar plate.
Incubate for 18-24 hours at 37°C and observe for the development of grey colonies surrounded by a brown halo (esculin hydrolysis positive). Re-incubate negative plates for additional 18-24 hours.
If the confirmation test is performed on colonies grown on filter membrane on enterococcal selective medium, transfer the membrane to Bile Aesculin Agar plate, incubate for 4 hours at 37°C and observe for the formation of a brown halo around the colonies.
The confirmation test can be completed by the catalase test by pouring a few drops of H_2O_2 over the colonies. Enterococci are aesculinase positive and catalase negative.

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, 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	37°C /18-24H-A	good growth, grey colonies surrounded by a brown halo
E. faecium ATCC 19434	37°C /18-24H-A	good growth, grey colonies surrounded by a brown halo

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

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 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Bile Aesculin Agar is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
The productivity characteristics are tested by semi-quantitative ecometric technique with the following target strains: *E.faecalis* ATCC 29212, *E.faecalis* ATCC 33186, *E. faecium* ATCC 19434, *E.hirae* ATCC 8043. After incubation at 37°C for 18-24 hours the target strains exhibit colonies with blackening of the medium.
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 following non-target strains: *S.aureus* ATCC 25923, *E.coli* ATCC 25922. The growth of the non-target strain is partially inhibited after incubation at 37°C for 18-24 hours

12 – LIMITATIONS OF THE METHOD

- Because strains of *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Vagococcus* with phenotypic similarities have been isolated from human infections, the presumptive identification of enterococci based only on Bile Esculin test and growth in 6.5% NaCl broth can be erroneous.[8]
- Bile Esculin test was originally used to identify the enterococci. However, other Group D Streptococci and occasionally non-Group D streptococci and other genera, *Aerococcus* and *Listeria* can tolerate the bile concentration and split aesculin. [6]
- Some *viridans* species (*S.sanguis*, *S.mutans* and *S.anginosus)* are capable of splitting aesculin but cannot usually tolerate an increased concentration of 4% bile and hydrolize aesculin in combination.[6]
- 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.
- It is advisable not to extend the incubation beyond 24 hours because the extensive blackening of the medium hinders reading.

13 - PRECAUTIONS AND WARNINGS

- This product 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. 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.
- 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 Sheet 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.

14 - STORAGE CONDITIONS AND SHELF LIFE

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 for the validation of the shelf life of the finished products, according to the type (plates/tubes/bottles) and the storage method (temperature and packaging).

15 - REFERENCES

1. Rochaix, A. Milieux a l'esculine pour le diagnostic differential des bacteries du groups strepto-enteropneumocoque. C R Soc Biol. 1924; 90:771-772.
2. Meyer K, Schonfeld H. Uber die Untersheidung des Enterococcus vom Streptococcus viridans and die Beziehunger beider zum Streptococcus lactis. Zentralbl Bakteriol Parasitenkd Infectionskr Hyg Abt Orig. 1926; 99:402-416.
3. Swan A. The use of a bile-esculin medium and of Maxted's technique of Lancefield grouping in the identification of enterococci (group D streptococci). J Clin Pathol 1954; 7:160-163.
4. APHA Standard Methods for the Examination of Water and Wastewater. 23th ed. American Public Health Association, Washington, D.C. 2017.
5. Manuel Suisse des Denrées Alimantaires (MSDA). Chapitre 56, Microbiologie. Juillet 2000
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
7. Lindel SS, Quin P. Use of Bile-Esculin Agar for Rapid Differentiation of Enterobacteriaceae J Clin Microbiol.1975; 1(5): 440-443
8. Teixeira LM et al. *Enterococcus*. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

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

TABLE OF APPLICABLE SYMBOLS

REF Or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/07

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