



AESCULIN BILE AZIDE AGAR

Dehydrated culture medium

Aesculin Bile Azide Agar: *Enterococcus faecalis*

1 - INTENDED USE

Selective and differential medium for the isolation and differentiation of enterococci from food, water and environmental samples.

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

Tryptone	17.00 g
Peptone	3.00 g
Yeast extract	5.00 g
Oxgall	10.00 g
Sodium chloride	5.00 g
Sodium citrate	1.00 g
Aesculin	1.00 g
Fe-Ammonium cCitrate	0.50 g
Sodium azide	0.25 g
Agar	13.00 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

Aesculin Bile Azide Agar is a selective and diagnostic medium for enterococci. Rochaix first reported in 1924¹ on the validity of the esculin hydrolysis reaction for the identification of enterococci. Media of this type have been studied and proposed since 1950, and some authors evaluated their efficacy in the clinical and hygienic field.²

Aesculin Bile Azide Agar is based on the formulation of Isenberg et al.³ who, in 1970, modified the Rochaix formula by reducing the concentration of bile salts and adding sodium azide for the isolation of group D-streptococci and their differentiation from non-group D streptococci. According to current bacterial nomenclature, the designation 'group D streptococci' for faecal streptococci is not to be regarded as specific since the Lancefield group D antigen is produced by *Enterococcus*, *Pediococcus* and some streptococci.^{4,5} Differentiation between group D-enterococci and group D non-enterococci (both are positive in the esculin test) can be made by the 6.5% NaCl tolerance test: the former group is tolerant to this salt concentration, the latter is inhibited by it.^{5,6}

Tryptone, peptone and yeast extract provide nitrogen, carbon, vitamins, amino acids and trace elements for microbial growth; sodium azide and bile salts are selective agents that limit the growth of Gram-negative and Gram-positive bacteria other than streptococci; esculin is hydrolysed by enterococci to glucose and esculetin (6-7-dihydroxycoumarin); esculetin reacts with the iron salts in the medium, giving it a brown-black colour.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 55.7 in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121° C for 15 minutes. Do not exceed sterilisation time and temperature. Mix well and distribute in sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared plates appearance	tan with trace blue cast, limp
Final pH at 20-25 °C	7.1 ± 0.2

6 - MATERIALS PROVIDED

Product	Type	REF	Pack
Aesculin Bile Azide Agar	Dehydrated medium	4010142	500 g (9 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, membrane filters, ancillary culture media and reagents for the complete identification of the colonies.

8 - SPECIMENS

Refer to applicable international standards and regulations for the collection of food and water samples. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

9 - TEST PROCEDURE

Prepare tenfold dilutions of the sample with peptone water. Within 3 hours from the sample preparation, spread 0.1 ml of the inoculum onto the plates. Incubate at 35°C or at 42°C for 18-24 hours (the higher incubation temperature increases the selectivity of the medium).

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies. The appearance of enterococci colonies on Aesculin Bile Azide Agar after 24 hours of incubation at 37°C is characteristic: convex, translucent or whitish colonies, 1-2 mm in diameter with dark brown or black halo; incubation should not be prolonged beyond 24 hours, because extensive blackening of the medium makes reading difficult.

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
<i>E. faecalis</i> ATCC 29212	37°C/24 H/A	good growth, colonies with blackening
<i>S. pyogenes</i> ATCC 19615	37°C/24 H/A	partially inhibited
<i>E. coli</i> ATCC 25922	37°C/24 H/A	inhibited

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

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Aesculin Bile Azide Agar is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch (RB).

The productivity characteristics are tested by semi-quantitative ecometric technique with the following target strains: *E. faecalis* ATCC 29212, *E. faecalis* ATCC 19433, *E. faecium* ATCC 19434, *E. hirae* ATCC 10541. 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. pyogenes* ATCC 19615, *S. pneumoniae* ATCC 6303, *S. aureus* ATCC 25923, *E. coli* ATCC 25922. The growth of the non-target strain is totally inhibited after incubation at 37°C for 18-24 hours

13 – LIMITATIONS OF THE METHOD

- 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.
- Other microorganisms such as *Listeria* and *Aerococcus* and occasionally strains of *S. mutans* and *S. sanguis* may grow on Aesculin Bile Azide Agar with blackening of the medium. The esculin hydrolysis test cannot be used alone to identify enterococci but used in combination with other tests: Gram staining, catalase, growth in the presence of 6.5% NaCl. Gram-positive, esculinase-positive, catalase-negative cocci that grow in the presence of 6.5% NaCl are enterococci.⁶
- There are streptococci that grow in the presence of sodium azide but do not hydrolyse esculin; the presence of growth in the absence of blackening is not an identifying characteristic of enterococci.⁶
- Media with esculin, bile salts and sodium azide are more suitable for primary isolation of enterococci than for their differentiation, for which culture media without sodium azide (Bile Esculin Agar) are better.
- Although the intended use and method of use of the medium described here refers to the detection of enterococci in food and other materials and therefore the product should not be regarded as an *in vitro* diagnostic, the literature reports the use of Bile Azide Agar for the detection of *Enterococcus* spp. in clinical specimens. Clinical applications should be validated by the user.
- 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.

14 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control only 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. This product is classified as dangerous: 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 the 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.

15 - 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.

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/flasks) and the applied storage conditions (temperature and packaging). According to MacFaddin⁶ the self-prepared plates can be stored at +2°C +8°C in the dark and protected against evaporation for up to four weeks.















16 - REFERENCES

1. Rochaix, A. Milieux a l'esculine pour le diagnostic differential des bacteries du groups strepto-enteropneumocoque. C R Soc Biol. 1924; 90:771-72.
2. 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; 7:160-3.
3. Isenberg HFD, Goldberg D, Sampson J. Laboratory studies with a selective Enterococcus medium. Appl Microbiol.1970;20:433-36.
4. Hardie D, J.M., Whiley R.A. Classification and overview of the genera Streptococcus and Enterococcus J App Microbiol Symposium Supplement, 1997,83, 1S-11S
5. <https://www.sciencedirect.com/topics/earth-and-planetary-sciences/streptococcus>
6. MacFaddin, JF. Media for Isolation, Cultivation, Identification, Maintenance of Medical Bacteria. Williams & Wilkins, Baltimore, MD, 1985.

TABLE OF APPLICABLE SYMBOLS

 Or  Catalogue number	 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 1	Updated layout and content	2022/04

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

