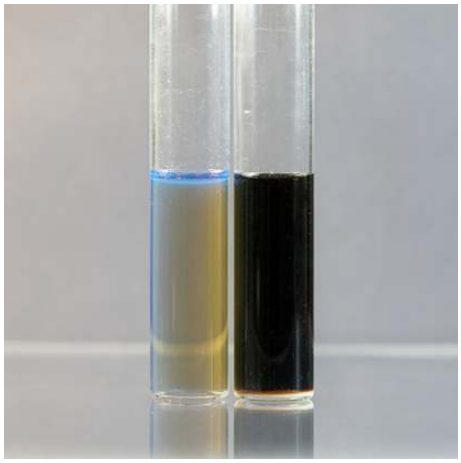
AESCULIN BILE AZIDE BROTH

Dehydrated culture medium



Aesculin Bile Azide Broth: unincoylated tube and *Enterococcus faecalis* growth

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 Citrate	0.50 g
Sodium Azide	0.25 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 Broth is a selective and diagnostic medium for enterococci. It differs from solid plated medium only in the omission of agar. Enterococci grow in the presence of sodium azide and bile salts and also have the ability to hydrolyse esculin to glucose and esculetin (6-7 dihydroxycoumarin). Rochaix first reported in 1924[1] 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 Broth 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; the authors reported the results of a study on 250 'D streptococci' strains from clinical isolation, showing that the medium was not inhibitory for the strains. They also observed that on this medium only *Listeria monocytogenes* among 86 strains belonging to various Gram-positive and Gram-negative bacterial species grew with an appearance similar to that of 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.

A more complete description of the characteristics of the faecal streptococci/intestinal enterococci group can be found in the data sheet of Azide Maltose Agar KF (401107).

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 42.7 g of broth in 1000 ml of cold distilled water. Heat to dissolve, distribute into screw capped tubes and sterilise by autoclaving at 121° C for 15 minutes. Do not exceed sterilisation time and temperature.

5 - PHYSICAL CHARACTERISTICS

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

6 - MATERIALS PROVIDED

Product	Type	REF	Pack
Aesculin Bile Azide Broth	Dehydrated medium	40101412	500 g (11.5 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops, swabs and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, tubes, ancillary culture media and reagents.

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 1 ml of the inoculum into the tubes. Incubate at 35°C or at 42°C for 18-24 hours (the higher incubation temperature increases the selectivity of the medium).

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10 - READING AND INTERPRETATION

The appearance of esculin-positive tubes is characterised by turbidity in the broth and the formation of a dark brown or black colour.

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	37°C/24 H/A	good growth, blackening of the broth
S.pyogenes ATCC 19615	37°C/24 H/A	partially inhibited
E.coli 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 Broth (TB:Test Batch) is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch (RB).
Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 35-37°C for 16-24 hours and recording the highest dilution showing growth in Reference Batch (GrRB) and in Test Batch (GrTB). Productivity is tested with the following target strains: *E.faecalis* ATCC 29212, *E.faecalis* NCTC 8132 *E.faecium* ATCC 19434, *E.gallinarum* ATCC 49573. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1. The growths exhibit the typical blackening of the broth.
Selectivity is tested with the following non-target strains: *S.pyogenes* ATCC 19615 *S.pneumoniae* ATCC 6303, *S.aureus* ATCC 25923, *E.coli* ATCC 25922. After incubation at 35-37°C for 24 hours, *E.coli* is totally inhibited while the growth of other non-target strains is partially inhibited.

13 – LIMITATIONS OF THE METHOD

- Other microorganisms such as *Listeria* and *Aerococcus* and occasionally strains of *S.mutans* and *S.sanguis* may grow on Aesculin Bile Azide Broth 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.

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 medium 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 (tubes/flasks) and the applied storage conditions (temperature and packaging).

16 - REFERENCES

1. Rochaix, A (1924) Milieux à l'esculine pour le diagnostic différentiel des bactéries du groupe strepto-entéro-pneumocoque. C.R. Soc. Biol. 90, 771.
2. Swan, A. (1954) - The use of a Bile, Aesculin Medium and of Maxted's technique of Lancefield grouping in the identification of enterococci (group D streptococci) J. Clin. Path, 7, 160-163.
3. Isenberg, H.D., Goldberg, D. & Sampson, J. (1970) - Laboratory studies with a selective enterococcus medium. Appl. Micr., 20, 433-436.
4. Hardie, D. J.M., Whiley R.A. (1997) Classification and overview of the genera Streptococcus and Enterococcus RealiJ. App. Microbiol. Symposium Supplement, 83, 1S–11S
5. https://www.sciencedirect.com/topics/earth-and-planetary-sciences/streptococcus
6. MacFaddin, Jean F. (1985). Media for Isolation, Cultivation, Identification, Maintenance of Medical Bacteria. Williams & Wilkins, Baltimore, MD

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 1	Updated layout and content	2022/04

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

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