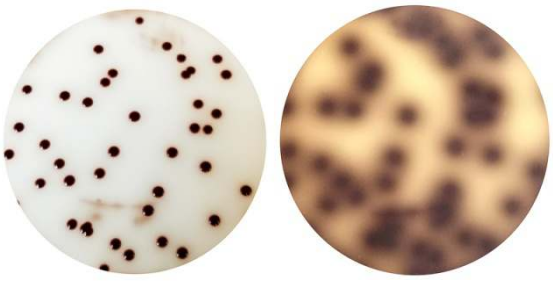
BILE AESCULIN AZIDE AGAR ISO FORMULATION

Dehydrated and ready-to-use culture medium



E.faecalis grown on Slantetz Bartley Agar (at left), transferred to BEEA-ISO and incubated for 2 hours (at right).

1 - Intended use

Selective and differential medium for the confirmation test of enterococci colonies according to ISO 7899-2.

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
Aesculin	1.00 g
Ferric ammonium citrate	0.50 g
Sodium azide	0.15 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

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. Isenberg, Goldberg and Sampson reduced the concentration of bile and added sodium azide.[3]
Bile Aesculin Azide Agar ISO Formulation meets the requirements described by ISO 7899-2 for the confirmation test of enterococci colonies isolated from water, based on their ability to grow and hydrolyse the glycoside aesculin.[4] 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. The method described by APHA for water samples requires Azide Dextrose Broth for MPN technique followed by a confirmatory streaking on Bile Aesculin Azide Agar.[5]
Tryptone, peptone and yeast extract provide nitrogen, carbon, amino acids, group B vitamins and trace elements for microbial growth; bile salts are selective agents that limit the growth of Gram-positive bacteria other than enterococci while sodium azide inhibits the growth of Gram-negative bacteria; esculin is hydrolysed by enterococci to glucose and aesculetin (6-7dihydroxycoumarin): aesculetin reacts with ferric ammonium citrate to form a dark brown or black complex.[6] Compared to Isenberg's classic formulation, the ISO medium contains less sodium azide and no sodium citrate.

4 - Directions for medium preparation

Suspend 54.7 g of 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. Cool to approximately 47-50 °C and transfer into sterile Petri dishes.

5 - Physical characteristics

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared medium appearance	tan with trace blue cast, limpid
Final pH at 20-25 °C	7.1 ± 0.1

6 - Materials provided - packaging

Product	Type	REF	Pack
Bile Aesculin Azide Agar ISO Formulation	Dehydrated medium	4010182	500 g (9 L)
Bile Aesculin Azide Agar ISO Formulation	Ready-to-use plates	541018	2 x 10 plates ø 90 mm
Bile Aesculin Azide Agar ISO Formulation	Ready-to-use plates	491018	3 x 10 plates ø 55 mm

7 - Materials required but not provided

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

8 – Specimens

The methods of analysis described here is suitable for the examination of drinking water, water from swimming pools and other clean or disinfected water. MF 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.[4] MPN method is recommended for drinking water, source water, and both fresh and marine recreational waters.[5] The application of the MF method appears particularly appropriate for the analysis of large quantities of water containing low number of intestinal enterococci. Refer to the cited Standards[4,5] for operational sampling details.

9 - test procedure

Membrane filter method[4]

1. Filter a suitable volume of sample through a 0.45 µm membrane filter.
2. Place the membrane on a Slanetz Bartey Agar plate (REF 402046) and incubate at 36 ± 2°C for 44 ± 4 hours.
3. After incubation, consider as typical all colonies that show 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 and incubate at 44 ± 0.5°C for 2 hours.

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MPN technique[5]

1. Inoculate a series of tubes of Azide Dextrose Broth (REF 401105) with appropriate graduated quantities of a 100 mL sample. Use sample volumes of 10 mL or less. The strength of the broth will be proportional to the sample size.
2. Incubate at 35 ± 0.5°C for 24 ± 2 hours and observe for microbial growth (turbidity of broth); if no turbidity is observed, continue incubation for a further 24 hours.
3. Streak a portion of growth from each positive tube on Bile Aesculin Azide Agar ISO Form. and incubate at 35°C for 24 ± 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 with a brown to black halo that had previously grown red-brown or pink on Slanetz Bartley Agar or showing turbidity in Azide Dextrose Broth.

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	44°C /2 H-A	good growth, colonies surrounded by a black halo
E. faecium ATCC 27270	44°C /2 H-A	good growth, colonies surrounded by a black halo

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 Bile Aesculin Azide Agar ISO Formulation are tested for productivity by comparing the results with a previously approved Reference Batch.
The productivity characteristics are tested by a quantitative technique on membrane filters placed on Slanetz Bartley Agar plates, incubated and then transferred on the surface of Bile Aesculin Azide Agar ISO Formulation plates, with the following target strains: *E.faecalis* ATCC 29212, *E.faecalis* ATCC 19433, *E. faecium* ATCC 27270, *E.faecium* ATCC 6057. After incubation at 44°C for 2 hours the target strains exhibit a blackening of the medium around the colonies.

13 – LIMITATIONS OF THE METHOD

- ISO 7899 describes a method for the isolation and enumeration of intestinal enterococci, mainly belonging to the species *Enterococcus 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.[4]
- *S.bovis* and *S.equinus* can be differentiated by further tests: growth at 45°C in BHI Broth, absence of growth in BHI Broth with 6.5% NaCl.[5]
- 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. 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 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

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°C /+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°C /+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).

16 - REFERENCES

1. Rochaix, A. Milieux a l'esculine pour le diagnostic differential des bacteries du groups strepto-enteropneumocoque. C R Soc Biol. 1924; 90:771-772.
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. Isenberg HFD, Goldberg D, Sampson J. Laboratory studies with a selective Enterococcus medium. Appl Microbiol.1970 Sep;20(3):433-6.
4. ISO 7899-2:2000 Water quality — Detection and enumeration of intestinal enterococci — Part 2: Membrane filtration method
5. APHA Standard Methods for the Examination of Water and Wastewater 23rd ed. Washington, DC: American Public Health Association, 2017.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

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 1	Updated layout and content	2022/05

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

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