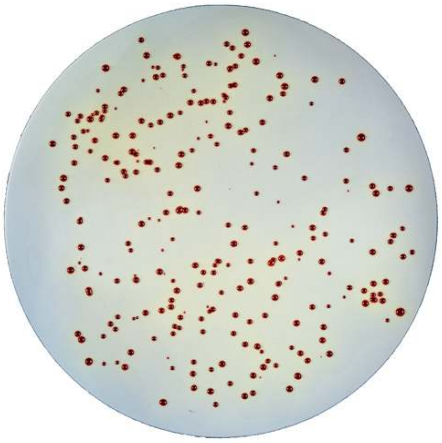
AZIDE MALTOSE AGAR KF

Dehydrated culture medium, ready-to-use plates



Azide Maltose Agar KF: *Enterococcus faecalis* colonies on a filter membrane

1 - INTENDED USE

For the enumeration of enterococci in water and food samples.

2 – COMPOSITION*

DEHYDRATED AZIDE MALTOSE AGAR KF

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptocomplex	10 g
Yeast extract	10 g
Sodium chloride	5 g
Sodium glycerophosphate	10 g
Maltose	20 g
Lactose	1 g
Agar	15 g
Sodium azide	400 mg
Bromocresol purple	15 mg

AZIDE MALTOSE AGAR KF, READY-TO-USE PLATES

Azide Maltose Agar KF	71.4 g
2,3,5-triphenyltetrazolium chloride	100 mg
Purified water	1000 mL

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Azide Maltose Agar KF, with added TTC is prepared according to the formula developed by Kenner, Clark and Kabler (Kenner-Faecal KF Medium)[1] for the selective isolation of faecal streptococci from surface water by direct inoculation or membrane filtration methods.
The medium is recommended by APHA[2] for the isolation and enumeration of enterococci in food samples by pour-plate technique.
The faecal streptococci group has long been considered an effective indicator of faecal contamination in aquatic ecosystems; although some authors consider the terms faecal streptococci, enterococci, intestinal enterococci and *Enterococcus* to be synonymous in the case of species detectable in the environment, the taxonomic ordering of the group has been the subject of numerous definitions.[3]
The term faecal streptococci has been used to denote a heterogeneous group of microorganisms both taxonomically and ecologically, grouped together on the basis of microscopic morphology, reactivity to Gram stain and the absence of catalase.
Studies in the 1980s subdivided the genus *Streptococcus*, on the basis of physiological characteristics and DNA hybridisation techniques, into three genetically different genera (*Enterococcus, Streptococcus, Lactococcus*), the first two of which include intestinal or faecal species.
The genus *Enterococcus* includes Lancefield group D streptococci, which share certain biochemical properties and have a wide range of tolerance to adverse conditions (ability to grow in 6.5% sodium chloride, pH 9.6 and 45°C); these phenotypic characteristics are, however, ascribable to most but not all species. The taxonomy of the genus is continually evolving: the genus, based on 16S rRNA sequence analysis, includes 79 species and 3 subspecies to date.[4]
Enterococci can be found in soil, water, dairy products, food and plants, and include species with a proven intestinal origin (*E. faecalis, E. faecium, E. durans/hirae, E. cecorum*) and others whose exclusively intestinal origin has not been fully proven (*E. raffinosus, E. dispar, E. flavescens, E. casseliflavus, E. gallinarum, E. mundtii, E. sulphureus*).[5-7]
Within the genus *Streptococcus*, only *S. bovis* and *S. equinus* are considered true faecal streptococci. These two streptococcus species are mainly found in animals.
The name Group D Streptococci, to denote faecal streptococci, is not to be trusted as the Lancefield Group D antigen is produced by *Enterococcus*, *Pediococcus* and some streptococci.
As a consequence of this taxonomic complexity and the multiple habitats of enterococci, more attention should be paid to their identification at the species level to discriminate those truly intestinal of animal, human and warm-blooded origin and their role as indicators of faecal pollution, for the assessment of the hygienic quality of water and food.
In Azide Maltose Agar KF, proteose peptone and yeast extract provide nitrogen, vitamins, amino acids and trace elements for microbial growth; lactose and maltose are fermentable carbohydrates: the production of acids induces the turning of the pH indicator bomocresol purple to yellow; sodium chloride contributes to the osmotic balance of the medium; sodium azide is a selective agent active in inhibiting the growth of Gram-negative bacteria; triphenyltetrazolium chloride added to the base medium is reduced during bacterial growth to formazan, an insoluble pigment that colours the colonies pink-red.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 71.4 g in 1000 mL of cold purified water; heat to boiling, and boil for five minutes (or autoclave 10 min at 121°C, if total selectivity is required). Cool to 50°C and aseptically add 10 mL of TTC 1% Solution (REF 42111801). Mix well and pour into sterile 55- or 90-mm Petri dishes or hold at 45°C when using the pour-plate method.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pink, limpid
Final pH at 20-25 °C	7.2 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Azide Maltose Agar KF	Dehydrated medium	4011072	500 g (7 L)
Azide Maltose Agar KF	Ready-to-use plates	491107	3 x 10 plates ø 55 mm

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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, TTC 1% Solution (REF 42111801) 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 water and food samples. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

9 - TEST PROCEDURE

Membrane filter technique

Filter an appropriate volume of water onto the membrane depending on the expected enterococci number. When the sample's bacterial density is unknown, filter several volumes or dilutions to achieve a countable plate (20-60 UFC/dish).
Using aseptic technique, roll the membrane filter used to collect the water sample onto the surface of the agar, so as to avoid the formation of air bubbles between the filter and the agar surface.
Incubate at 35-37°C for 48 hours.

Pour-plate method[2]

Place 1 mL of the decimal dilutions of the sample in 90 mm diameter plates, in duplicate;
Add approximately 15 mL of medium cooled to 45°C to each plate, carefully mix the inoculum with the agar and allow to solidify.
Incubate at 35-37°C for 48 hours.

10 - READING AND INTERPRETATION

After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies, if necessary, using a 15x stereoscopic microscope. Count and record pink to red colonies often surrounded by a yellow halo as enterococci.

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 19433	37°/ 48 H-A	Growth with pink-red colonies with yellow halo
E. faecium ATCC 19434	37°/ 48 H-A	Growth with pink-red colonies with yellow halo
S. aureus ATCC 25923	37°/ 48 H-A	Inhibited
E. coli ATCC 25922	37°/ 48 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 Azide Maltose Agar KF 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 19433, *E. faecium* ATCC 19434, *E. hirae* ATCC 8043, *E. casseliflavus* CB ENT7.1, *E. gallinarum* ATCC 49573, *S. bovis* ATCC 33317. After incubation at 37°C for 48 hours the amount of growth and the colony characteristics are evaluated: target strains exhibit good growth with pink-red colonies.
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. salivarius* ATCC 7073, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *L. acidophilus* ATCC 314. The growth of the non-target strain is totally inhibited after incubation at 37°C for 48 hours
Note: CB: Biolife culture collection

13 – LIMITATIONS OF THE METHOD

- KF Agar was found to be unsuitable for marine water because *Vibrio alginolyticus* and other Gram-negative bacilli indigenous to this environment grew well on it and produced red colonies identical to those of faecal streptococci.[8]
- Many strains of *S. bovis* and *S. equinus* are inhibited by sodium azide.
- The intensity of TTC reduction varies depending of the isolated species.[2]
- Most but not all enterococci and streptococci ferment lactose and sucrose.[2]
- Some strains of *Pediococcus*, *Lactobacillus* and *Aerococcus* may grow on the medium, producing light pink colonies.[2]
- For the examination of dairy products, a more selective medium and a higher incubation temperature should be used to reduce the background growth of lactobacilli and lactic streptococci.[2]
- Over-heating of the medium lowers pH resulting in a decreased productivity.[9]
- 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

- Azide Maltose Agar KF 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. Dehydrated Azide Maltose Agar KF is classified as dangerous since contains sodium azide which tends to form explosive metal azides with plumbing materials. 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 medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the inoculated plates 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 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

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).

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 (plates/flasks) and the applied storage conditions (temperature and packaging). According to MacFaddin the medium base without TTC may be stored in screw capped flasks at +2°C /+8°C for approximately 6 months.[9]

16 - REFERENCES

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9. 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	For single use only	This side up	Store in a dry place
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Fragile	Keep away from direct light

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2023/01

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

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