AZIDE DEXTOSE BROTH

Dehydrated and ready-to-use culture medium

Azide Dextrose Broth; from left: uninoculated tube and tube with *Enterococcus faecium*.

1 - INTENDED USE

Selective liquid medium for the detection of faecal streptococci/enterococci in water, frozen food, milk and other samples.

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

Peptocomplex	15.0 g
Beef extract	4.5 g
Glucose	7.5 g
Sodium chloride	7.5 g
Sodium azide	0.2 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

Enterococci are considered a better indicator of sewage contamination than *Escherichia coli* as they are more resistant to chlorine.
Azide Dextrose Broth is a medium initially formulated by Rothe[1] and tested by Mullmann and Seligmann[2] for the quantitative determination of streptococci in water, wastewater, shellfish and other materials with suspected faecal contamination. Azide Dextrose Broth has also been used for the isolation of streptococci in food, milk, water and other samples of sanitary interest as indicators of faecal contamination.[3,4]
Azide Dextrose Broth is used for the presumptive determination of enterococci in water and wastewater using the MPN technique followed by confirmation test in Bile Aesculin Azide Agar or Ethyl Violet Azide Broth.[5-8]
A similar procedure is indicated in the APAT, IRSA-CNR guidelines[9] for the detection of enterococci in water using the MPN method.
Peptocomplex and meat extract provide nitrogen, amino acids and trace elements for microbial growth; sodium azide limits the growth of Gram-negative bacteria by blocking the enzyme cytochrome oxidase; glucose is a fermentable carbohydrate; sodium chloride contributes to maintaining the osmotic balance of the medium

4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 34.7 g in 1000 mL of cold purified water. Heat gently to dissolve, distribute 10 mL into tubes and sterilise by autoclaving at 121°C for 15 minutes. For inocula of more than 1 mL per 10 mL medium prepare the liquid medium at double or multiple concentration.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Prepared tubes appearance	straw-coloured, limpid
Final pH at 20-25 °C	7.2 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Azide Dextrose Broth	Dehydrated medium	4011052	500 g (14.5 L)
Azide Dextrose Broth	Ready-to-use tubes	551105	20 x 10 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 – SPECIMENS

Drinking water, source water, fresh and marine recreational waters and food samples. Refer to applicable International Standards and regulations for the collection, transport, storage and preparation of samples and operate in accordance with good laboratory practice.

9 - TEST PROCEDURE

1. Inoculate a series of tubes of Azide Dextrose Broth 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. (REF 401018) and incubate at 35°C for 24 ± 2 hours.[6]
4. Alternatively, remove 1 mL of broth culture from the positive tubes and inoculate into the corresponding tubes containing Ethyl Violet Azide Broth (REF 401485) for confirmation testing. Incubate the tubes at 35 °C for 24+24 (±3) hours.

10 - READING AND INTERPRETATION

Bacterial growth in Azide Dextose Broth is evidenced by the development of turbidity.
After confirmation tests, apply MPN tables for estimating the number of faecal streptococci per volumetric unit of sample.

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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°C /24H-A	good growth
E. faecium ATCC 19434	37°C /24H-A	good growth
E. coli ATCC 25922	37°C /24H-A	inhibited

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 Azide Dextrose Broth (TB: Test Batch) is assessed 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 37°C for 24 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following target strains: *E.faecalis* ATCC 29212*, E..faecalis* ATCC 19433, *E.faecium* ATCC 19434, *E.hirae* ATCC 10541. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.
Selectivity is tested with the following non-target strains: *S.aureus* ATCC 25923, *E.coli* ATCC 25922, *B.cereus* ATCC 11778. After incubation at 37°C for 24 hours, the growth of *E.coli* and *B.cereus* is totally inhibited while the growth of *S.aureus* is partially inhibited.

13 – LIMITATIONS OF THE METHOD

- Since some Gram-positive bacilli and cocci other than faecal streptococci grow in Azide Dextrose Broth, a confirmation test is required.

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 Material Safety Data Sheets.
- 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.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.
- Ready-to-use tubes are subject to terminal sterilization by autoclaving.
- Each tube of this culture medium is for single use only.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder 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 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 medium in tubes

Upon receipt, store tubes in their original pack at 2-8°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. Tubes from opened secondary packages can be used up to the expiration date. Opened tubes must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

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

16- REFERENCES

1. Rothe (1948) Illinois State Health Department.
2. Mallmann WL, Seligmann A. comparative study of media for the detection of streptococci in water and sewage. Am J Public Health 1950; 40:286
3. Larkin, EP, Litsky, W, Fuller JE. Fecal streptococci in frozen foods I A bacteriological survey of some commercially frozen foods. Appl Microbiol 1955; 3:98-101
4. Splittstoesser DF, Wright R, Hucker GJ. Studies on Media for Enumerating Enterococci in Frozen Vegetables. Appl. Microbiol. 1962; 9:303.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985

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6. APHA Standard Methods for the Examination of Water and Wastewater 23rd ed. Washington, DC: American Public Health Association, 2017.
7. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington, D.C. 1976
8. WHO Examination of water for pollution control. Part III: Biological, Bacteriological and Virological Examination., ed. Oxford. Pergamon Press, World Health Organization.1982
9. APAT, IRSA-CNR Manuali e Linee Guida 29/2003 Metodi analitici per le acque. Cap 3, 7040.

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 2	Updated layout and content	2022/05

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

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