AZIDE DEXTOSE BROTH (ROTHE)

Dehydrated culture medium

1 - INTENDED USE

Selective medium for the detection of enterococci in water and sewage.

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

Peptone	20.0 g
Glucose	5.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.7 g
Potassium dihydrogen phosphate	2.7 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

Azide Dextrose Broth (Rothe) is a buffered version of the medium initially formulated by Rothe at the Illinois State Health Department.[1]
The presence of enterococci is an indicator of faecal contamination mainly in chlorinated water because they have a greater resistance to chlorine than *Escherichia coli*.
The peptone provides nitrogen, amino acids and trace elements for microbial growth, glucose is a fermentable carbohydrate; sodium azide limits the growth of Gram-negative bacteria by blocking the enzyme cytochrome oxidase; sodium chloride contributes to maintaining the osmotic balance of the medium; potassium phosphates buffer the medium.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 35.6 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 media at double or multiple concentration.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Prepared tubes appearance	pale yellow, limpid
Final pH at 20-25 °C	6.8 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Azide Dextrose Broth (Rothe)	Dehydrated medium	4011062	500 g (14 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 – SPECIMENS

Water and sewage and other samples. Refer to applicable International Standards and regulations for the collection, transport, storage of samples and operate in accordance with good laboratory practice.

9 - TEST PROCEDURE

1. Shake the sample vigorously to ensure homogeneous distribution of the suspended microorganisms.
2. Perform counting in tubes, according to the most probable number method (MPN); vary the size of the inoculum (multiples or fractions of 1 mL) according to the type of sample, setting up at least five tubes for each dilution.
3. Incubate at 36 ±1°C for 24 hours and observe for microbial growth (turbidity of broth); if no turbidity is observed, continue incubation for a further 24 hours.
4. Remove 1 mL of broth culture from the positive tubes and inoculate into the corresponding tubes containing Ethyl Violet Azide Broth (REF 401484) for confirmation testing.
5. Incubate the tubes at 36 ±1°C for 24+24 (±3) hours. Consider tubes with turbidity accompanied by a violet-grey deposit at the bottom of the tube as positive.
6. Other media may be used for the confirmation test; consult the cited bibliography and other applicable literature.

10 - READING AND INTERPRETATION

Bacterial growth in Azide Dextose Broth (Rothe) 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.

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	35-37°C /24H-A	good growth
E. coli ATCC 25922	35-37°C /24H-A	inhibited

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

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12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale representative samples of all lots of dehydrated and ready-to-use Azide Deztrose Broth Rothe (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: Group B *Streptococcus* ATCC 12386, *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* and Group B *Streptococcus* is partially inhibited.

13 – LIMITATIONS OF THE METHOD

- Since some Gram-positive bacilli and cocci other than faecal streptococci grow in Azide Dextrose Broth (Rothe), a confirmation test in Ethyl Violet Azide Broth or other suitable medium is required.

14 - PRECAUTIONS AND WARNINGS

- This product 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.
- 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 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.

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	2022/05

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

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