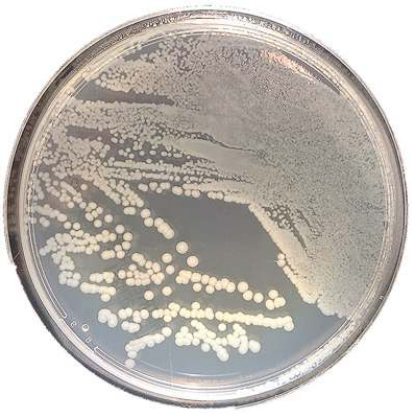
INSTRUCTIONS FOR USE

NUTRIENT AGAR

Ready-to-use flasks



Salmonella Typhimurium on Nutrient Agar

1 - INTENDED USE

In vitro diagnostic. General purpose medium for the cultivation, sub-culture and purification of colonies of non-fastidious microorganisms, isolated from clinical and non-clinical specimens.

2 - COMPOSITION -TYPICAL FORMULA *

Beef Extract	3 g
Peptone	5 g
Agar	15 g
Purified water	1000 mL

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Nutrient Agar is a culture medium based on meat peptones, used for the cultivation of non-fastidious microorganisms. The peptones provide carbon, nitrogen and vitamins for the growth of most non-fastidious microorganisms (e.g. enterobacteria, staphylococci). The absence of sodium chloride limits the swarming of Proteus spp. The formulation of the Nutrient Agar complies with the recommendations of ISO 6579[1] and ISO 10273[2]. Nutrient Agar was one of the first media utilised in microbiology and can still be used for the examination of water and food for preparing stock cultures, for the preliminary cultivation of a sample undergoing successive bacteriological examinations, and for the isolation of microorganisms in pure culture.

4- METHOD OF PREPARATION

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour into sterile Petri dishes or tubes, under aseptic conditions.

5 - PHYSICAL CHARACTERISTICS

Medium appearance	very pale yellow, limpid
Final pH at 20-25°C	7.0 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Nutrient Agar	Ready-to-use flasks	5118102	6 x 100 mL; 6 glass bottles with flat bottom and aluminium screw-cap; packaging: cardboard box.

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water bath or hot plate, incubator and laboratory equipment as required, sterile plastic Petri dishes, sterile tubes, sterile loops, needles and swabs, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Generally, Nutrient Agar is used for the sub-culture of microorganisms isolated on other culture media and is not used for the direct inoculation of clinical samples.

9- TEST PROCEDURE

Allow plates and or the tubes to come to room temperature and to dry the surface of the plated medium.

Plates

Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Routinely, incubate at 35-37°C in aerobic conditions for 18-24 hours.

The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the organisms to be cultivated and the local applicable protocols.

Tubes

For the subculture of colonies, by means of a sterile needle or loop, inoculate the slant with a colony cultivated on another isolation medium. Usually, an incubation temperature of 35-37°C C for 18-24 hours is adequate for cultivation of common aerobes and facultative anaerobes. The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the inoculated organism and the local applicable protocols.

10 - READING AND INTERPRETATION

The presence of microorganisms is indicated by the appearance of colonies of various morphology and size. The characteristics of the growths are closely related to the type or types of cultivated microorganisms.

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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
S.Typhimurium	ATCC	14028	35-37°C / 18-24H / A	good growth
E.coli	ATCC	25922	35-37°C / 18-24H / A	good growth
Y.enterocolitica	ATCC	23715	29-31°C / 18-24H / A	good growth

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 ready-to-use flasks and of the raw material used for the production, (dehydrated Nutrient Agar) is tested for productivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by a quantitative method with the target strains *E.coli* ATCC 25922*, S.*Typhimurium ATCC 14028*,Y.enterocolitica* ATCC 23715; Nutrient Agar plates are inoculated with decimal dilutions in saline of the colonies' suspensions and incubated at 35-37°C (*E.coli,* S.Typhimurium) and at 29-31°C (*Y.enterocolitica)* for 18-24 hours. The colonies are enumerated on Test Batch (TB) and Reference Batch (RB) and the productivity ratio (*Pr*=CFU_{TB}/CFU_{RB}) is calculated. If *Pr* is ≥ 0.7 the results are considered acceptable and conform to the specifications.
Productivity is also tested by semi-quantitative ecometric technique with *E.faecalis* ATCC 19433 and *S.aureus* ATCC 6538. After incubation at 35-37°C for 18-24 hours the amount of growth is evaluated and recorded. All strains show a good growth, comparable with the Reference Batch.

13 - LIMITATIONS OF THE METHOD

- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- 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 product 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Be careful when opening screw cap flasks to prevent injury due to breakage of glass.
- When using a hot plate and/or a water bath, boil sufficiently long to dissolve the whole medium.
- Wear heat-protective gloves during medium liquefaction. Do not place the hot flasks into an ice bath or in cold water to accelerate cooling as this might cause cracks in the glass.
- The time required for complete liquefaction of the medium may vary considerably and depends on the actual temperature of the heating device, its wattage, the size and volume of the bottle.
- Once the bottled medium is liquefied, it cannot be solidified and dissolved a second time.
- Ready-to-use flasks of Nutrient Agar are subject to terminal sterilization by autoclaving.
- 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 flasks in their original pack at 2-8°C away from direct light. If properly stored, the flasks may be used up to the expiration date. Do not use the flasks beyond this date. Flasks from opened secondary packages can be used up to the expiration date. Opened flasks must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use flasks with signs of deterioration (e.g. microbial contamination, abnormal turbidity, precipitate, atypical colour).
The user is responsible of the correctness of plates and/or tubes preparation. The user is responsible of the validation of tubes and plates shelf-life, according to the method of storage (temperature and packaging).

16 - REFERENCES

1. ISO 6579-1:2017 Microbiology of the food chain -- Horizontal method for the detection, enumeration and serotyping of Salmonella -- Part 1: Detection of Salmonella spp.
2. ISO 10273:2017 Microbiology of the food chain -- Horizontal method for the detection of pathogenic Yersinia enterocolitica.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content in compliance with IVDR 2017/746	2021/09
Revision 2	Removal of obsolete classification	2023/04

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

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