NUTRIENT GELATIN

Dehydrated culture medium

1 - INTENDED USE

For the differentiation of microorganisms on the basis of gelatinase production and for the 20°C plate count.

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

Beef extract	3 g
Peptone	5 g
Gelatin	120 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

Gelatin was used to obtain the first solid medium in 1881 by Robert Koch. Its digestion by bacteria and melting temperature at 37°C limited its use and it soon paved the way for agar, which has far superior material qualities.[1]
Nutrient Gelatin is prepared according with the formula formerly used in the examination of water, sewage, and other materials of sanitary importance.[2] The medium is mainly used to determine the ability of an organism to producer proteolytic-like enzymes (gelatinases) to liquefy gelatin, a standard method in taxonomic studies, and for water microbial plate count at 20°C.[3]
The peptone and beef extract provide nitrogen and minerals sufficient for the growth of non-fastidious organisms. The gelatin is the substrate for the determination of gelatinase.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 128 g in 1000 mL of cold purified water, leave for 10 minutes then bring to the boil to dissolve completely. Distribute and sterilise by autoclaving at 121 °C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution appearance	clear to slightly opalescent, may have a slight precipitate.
Final pH at 20-25 °C	6.8 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Nutrient Gelatin	Dehydrated medium	4018202	500 g (3.9 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Petri dishes, tubes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Gelatinase test: the sample consists of pure cultures of bacteria. Plate count: water, sewage, and other materials of sanitary importance.

9 - TEST PROCEDURE

Liquefaction test[3]
Inoculate a test tube of Nutrient Gelatin solidified at 4 °C with a drop of heavy microbial suspension by stabbing in centre of medium to a depth of approximately one-half an inch from the bottom of the tube. Set up a control uninoculated tube.
Incubate tubes at 35 ± 2°C °C for 24-48 hours and up to 14 days.
At the end of each 24 hours period, place all tubes in the refrigerator or in an ice bath for a sufficient period of time (approximately 2 hours) to determine whether digestion of gelatin (liquefaction) has occurred. Make transfer from incubator to refrigerator without shaking the tubes.
Invert tubes to test solidification; interpret reaction as soon as control tube has hardened.
If the microorganisms being examined contain proteolytic enzymes which hydrolyse gelatin, the medium remains liquid.
Standard plate count of water[4]
Dilute the sample with sterile water and place 0.5 or 1mL of the dilutions in each dish of at least two duplicate sets of sterile Petri dishes. Cool the Nutrient Gelatin to around 42 °C and aseptically add 10 mL to each dish. Mix the medium with the inoculum, solidify as soon as possible after pouring, and immediately place in an incubator at 19-21 °C. Incubate for 48 hour and count at least two plates containing between 30 and 300 colonies.

10 - READING AND INTERPRETATION

Positive liquefaction test
Test organism: medium liquefied / Control tube: medium remains solid
Negative liquefaction test
Test organism: medium remains solid
Reincubate for an additional period
Control tube: medium remains solid

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, it is responsibility of the end-user to perform Quality Control testing 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 of un-supplemented medium.

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CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S.marcescens ATCC 8100	20 ± 1°C / 44-48 H / A	growth, gelatinase positive
E.coli ATCC 25922	20 ± 1°C / 44-48 H / A	growth, gelatinase negative

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

12 - LIMITATIONS OF THE METHOD

- Gelatin is solid when incubated at 20°C or less and liquid at a temperature of 35°C or greater.
- Nutrient Gelatin is not recommended for determination of gelatin liquefaction by fastidious species and obligate anaerobes.
- Growth and liquefaction often only occur in the surface layer of the tube. To avoid false negative results, handle test tubes carefully when warm, so that the liquefied gelatin remains on the surface of the test tube and does not mix with the lower layers.[3]
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

13 - 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.

14 - 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 (plates/tubes/bottles) and the storage method (temperature and packaging).

15 - REFERENCES

1. Nabajit Das, Naveen Tripathi, Srijoni Basu, Chandra Bose, Susmit Maitra, and Sukant Khurana. Progress in the development of gelling agents for improved culturability of microorganisms. Front Microbiol. 2015; 6: 698.
2. American Public Health Association. 1960. Standard methods for the examination of water and sewage, 9th ed. American Public Health Association, New York, N.Y.
3. MacFaddin, Jean F. (1985). Media for Isolation, Cultivation, Identification, Maintenance of Medical Bacteria. Williams & Wilkins, Baltimore, MD.
4. American Public Health Association. 1946. Standard Methods for the Examination of Water and Sewage, 5th ed. American Public Health Association, New York, N.Y.

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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