

GLUCOSE OF MEDIUM

Dehydrated and ready to use culture medium



1 - INTENDED USE

Semi-solid medium for the confirmation test of *Enterobacteriaceae*.

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

Enzymatic digest of casein	2.00 g
Dipotassium hydrogen phosphate	0.30 g
Sodium chloride	5.00 g
Glucose	10.00 g
Bromothymol blue	0.08 g
Agar	3.00 g

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

Glucose OF Medium - On the left, a test tube inoculated with *E. coli*, on the right, a non-inoculated tube.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Glucose OF Medium is based on the formulation described by Hugh and Leifson¹ and meets the requirements of ISO 21528^{2,3}. The fermentation of glucose, together with oxidase test, is recommended by ISO 21528 for the confirmation of *Enterobacteriaceae* colonies isolated on Violet Red Bile Glucose Agar.

The medium contains glucose as fermentable carbohydrate and bromothymol blue as a pH indicator: the high concentration of acid produced during fermentation will turn the bromothymol blue indicator from green to yellow in the presence or absence of oxygen. The persistence, after incubation, of a green colour or the appearance of a blue colour, due to an alkaline transformation of the medium, indicates that there was no degradation of glucose. Glucose OF Medium is a semi-solid medium: the presence of agar at a concentration of 0.3% enables the determination of motility in addition to fermentation test and also aids in preventing the distribution of any acid produced towards the surface of the medium, with a consequent dilution. Dipotassium phosphate promotes carbohydrate fermentation and acts as a pH control buffer; enzymatic digest of casein provides carbon, nitrogen and trace elements for microbial growth; sodium chloride maintains the osmotic balance.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Dissolve 20.4 g in 1000 mL of cold purified water. Heat to boiling to completely dissolve the medium, dispense 10 mL into tubes and sterilize by autoclaving at 121 °C for 15 minutes. Allow the tubes to solidify vertically.

Before use, if necessary, heat the medium in boiling water or steam for 15 minutes to remove the oxygen present and then cool rapidly to the incubation temperature.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	green, fine, homogeneous, free-flowing powder
Solution and prepared tube appearance	green, clear
Final pH at 20-25 °C	6.8 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Glucose OF Medium	Dehydrated medium	4015252	500 g (24.5 L)
Glucose OF Medium	Ready-to-use tubes	551525	20 x 10 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Erlenmeyer flasks, tubes, paraffin oil, ancillary culture media and reagents.

8 - SPECIMENS

Pure culture of bacterial strains under examination.

9 - TEST PROCEDURE

Colonies of presumptive *Enterobacteriaceae* isolated on Violet Red Bile Glucose are sub cultured on a non-selective medium (Nutrient Agar) and then confirmed by means of oxidase test and the fermentation of glucose.

Using a wire, stab the colonies that gave a negative oxidase test into tubes containing Glucose OF medium.

Overlay the surface of the medium with minimal 1 cm of sterile mineral oil.

Incubate the tubes at 37 ± 1 °C for 24 h ± 2 h.

10 - READING AND INTERPRETATION

Positive reaction (acid formation) is indicated by a yellow colour throughout the content of the tube.

Negative reaction: the medium remains green or changes to blue.

If the colonies are oxidase-negative and glucose-positive, the sample shall be regarded as being positive for *Enterobacteriaceae*.

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 some test strains useful for the quality control of the medium overlaid with paraffin oil.

CONTROL STRAINS	INCUBATION T°/ T –ATM	EXPECTED RESULTS
<i>E. coli</i> ATCC 25922	37°C x 24 h-A	growth with colour change to yellow
<i>P. aeruginosa</i> ATCC 27852	37°C x 24 h-A	growth with absence of colour change or yellow only at the top of the tube.

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

12 – PERFORMANCES CHARACTERISTICS

Prior to release for sale, representative sample of all lots of dehydrated and ready to use Glucose OF Medium is tested for specific colour change of the tubes by comparing the results with a previously approved Reference Batch.

The medium is tested by inoculation the tubes overlaid with paraffin oil with pure culture of glucose fermenting strains (*E. coli* ATCC 25922, *E. aerogenes* ATCC 13048, *S. Typhimurium* ATCC 14028) and non-glucose fermenting strains (*P. aeruginosa* ATCC 27853, *P. fluorescens* ATCC 13525, *A. calcoaceticus* ATCC 19606). After incubation at 37°C for 24 hours the strains exhibit the following reactions:

Glucose fermenting strains: the medium turns to yellow.

P. aeruginosa and *P. fluorescens*: no colour change of the medium (yellow colour only at the top of the tube).

A. calcoaceticus: no colour change of the medium.

13 – LIMITATIONS OF THE METHOD

- Carbohydrate fermentation is one of the tests used to identify pure bacterial cultures. For complete identification, other suitable tests must be carried out.

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 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.
- 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 ready-to-use 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 Sheets of the products 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°C / +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 the validation of their shelf life, according to the type (plates/tubes/bottles) and the applied storage conditions (temperature and packaging). According to ISO 21528 the prepared tubes may be stored for up to 4 weeks at 2-8 °C.^{2,3}














16 – REFERENCES

- Hugh R, Leifson E. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. *J Bacteriol* 1953; 66:24-26
- ISO 21528-1:2017. Microbiology of the food chain — Horizontal method for the detection and enumeration of Enterobacteriaceae —Part 1: Detection of Enterobacteriaceae
- ISO 21528-2:2017. Microbiology of the food chain — Horizontal method for the detection and enumeration of Enterobacteriaceae — Part 2: Colony-count technique





TABLE OF APPLICABLE SYMBOLS

 or  Catalogue number	 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	2022/08

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

