TRYPTIC GLUCOSE EXTRACT AGAR

Dehydrated culture medium

1 - INTENDED USE

For microbial plate counts in milk, dairy products, water, and other samples of sanitary importance.

2 – COMPOSITION*

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	5.0 g
Beef extract	3.0 g
Glucose	1.0 g
Agar	15.0 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

In 1935, Bower and Hucker devised the composition of a medium (Tryptone Glucose Agar) for the bacteriological analysis of milk and reported higher plate counts and larger colony sizes.[1,2] Tryptic Glucose Extract Agar (TGEA) was originally suggested by the American Public Health Association in 1948[3] for the estimation of total viable counts in milk and dairy products and was later adopted for the analysis of water[4]. TGEA for many years remained the standard culture medium for microbial plate count. Currently it is recommended in the Compendium of Methods for the Microbiological Examination of Foods for performing the plate count of mesophilic aerobic endospore-forming bacilli[5] and for aerobic or heterotrophic plate count in bottled water[6].
Tryptone and beef extract provide nitrogen, carbon, minerals and amino acids for the microbial growth. Glucose is a source of carbon and energy; agar is the solidifying agent.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM

Suspend 24 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	pale beige, clear
Final pH at 20-25 °C	7.0 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Tryptic Glucose Extract Agar	Dehydrated medium	4021442	500 g (20.8 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile pipettes and spreaders, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, ancillary culture media and reagents.

8 – SPECIMENS

Milk, dairy products, water, and other samples of sanitary importance. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

Colony count by the pour plate technique.

1. Using a sterile pipette, dispense 1 mL of the liquid test sample, or 1 mL of an initial suspension in the case of other products, into an empty Petri dish and mix with the molten Tryptic Glucose Extract Agar pre-cooled to 44-46°C.
2. Prepare the other plates under the same conditions using decimal dilutions of the test sample or of the initial suspension.
3. Incubate the plates under aerobic conditions at 35 °C for 72 hours.

Colony count by the surface plating technique.

1. Dry the prepared plates before the use.
2. Using a sterile pipette, transfer 0.1 mL of the test sample, if the product is liquid, or of the initial suspension in the case of other products, to the centre of a Tryptic Glucose Extract Agar plate.
3. Carefully spread the inoculum uniformly and as quickly as possible over the surface of the agar plate, without touching the sides of the dish with the spreader.
4. Leave the plates with the lids on for about 15 min at ambient temperature for the inoculum to be absorbed into the agar.
5. Incubate the plates under aerobic conditions at 35 °C for 72 hours.

Consult the references for information regarding the details of processing and inoculation of bottled water samples[4] and the plate count of mesophilic aerobic endospore-forming bacilli[5].

10 - READING AND INTERPRETATION

After incubation, count all colonies obtained in the plates containing fewer than 300 colonies and calculate the number of microorganisms per gram or per millilitre of the test sample. Follow recommended procedures for the counting of colonies and the reporting of results.

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. coli ATCC 8739	37°C/72H/A	good growth
S. aureus ATCC 6538	37°C/72H/A	good growth
B. subtilis ATCC 6633	37°C/72H/A	good growth

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

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, e-mail: export@biolifeitaliana.it;
web: www.biolifeitaliana.it

12 – Performance characteristics

Prior to release for sale, a representative sample of all lots of dehydrated Tryptic Glucose Extract Agar is tested for productivity by comparing the results with Tryptic Soy Agar.
The productivity is tested by a quantitative method with the following strains *E. coli* ATCC 8739, *S. aureus* ATCC 6538 and *B. subtilis* ATCC 6633. The plates are inoculated by surface plating technique with decimal dilutions in saline of a colonies' suspension and incubated at 37°C for 24 hours. The colonies are enumerated on both media and the productivity ratio (Pr: CFU_{TGEA}/CFU_{TSA}) is calculated. If Pr is ≥ 0.7 the results are considered acceptable and conform to the specifications. Moreover the productivity characteristics are tested by semi-quantitative ecometric technique with the following strains: *S. pyogenes* ATCC 19615, and *E. faecalis* ATCC 19433, *L.acidophilus* ATCC 314. After incubation, the amount of growth is evaluated: the tested strains exhibit good growth.

13-Limitations of the methods

- A delay of more than 10 minutes between sample dispensing into Petri dishes and agar addition can result in lower counts.[7]
- A potential source of error in plate count can result from the stack-pouring Petri dishes: in a stack of 3 plates, the middle and the top plates took too longer to cool, thereby resulting in lower counts. [7]
- Increasing the holding time of the dilutions in the diluent leads to higher count. [7]
- The Aerobic Plate Count does not differentiate between different type of bacteria. Alteration in incubation time and temperature and the type of atmosphere will change the types of organisms that will grow and thus be counted.[7]

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 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 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 the validation of their shelf life, according to the type (plates/tubes/bottles) and the applied storage conditions (temperature and packaging).

16 – References

1. Bowers CS and Hucker GY. 1935. The composition of media for the bacteriological analysis of milk. Tech Bull. N.Y. State Agr. Expt. Sta. No. 228. 1935.
2. Bowers CS and Hucker GY. 1936. Further studies of the composition of media for the bacteriological analysis of milk. Am. J. of Public Health. 1936; 26:350–352
3. APHA Standard Methods for the Examination of Dairy Products. American Public Health Association, New York, N.Y. 9th ed. 1948.
4. APHA Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, D.C. 15th ed. 1980
5. Stevenson KE, Lembke F. Mesophilic Aerobic Endospore-Forming Bacilli. In: Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.
6. Posnick L, Feng P. Bottled Water. In: Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015
7. Petran R, Grieme LE, Foong-Cunningham S. Culture Methods for Enumeration of Microorganisms. In: Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.

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	2022/12

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