TRYPTIC GLUCOSE YEAST AGAR
(PLATE COUNT AGAR)
(STANDARD METHODS AGAR)

Dehydrated and ready-to-use culture medium

1 - INTENDED USE
For microbial plate counts in foodstuffs, milk, dairy products, water, and other samples of sanitary importance.

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

DEHYDRATED AND READY-TO-USE MEDIUM

Tryptone	5.0 g
Yeast extract	2.5 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
Tryptic Glucose Yeast Agar also known as Plate Count Agar or Standards Methods Agar is recommended by regulatory authorities[1-7] for the enumeration of mesophilic or thermophilic aerobic organisms in foodstuffs, milk, dairy products, water, raw materials and other samples of sanitary importance, and for the evaluation of sanitary conditions of environmental samples in the area of food and feed production, and handling.
This test is based on the assumption that each viable cell, pair of cells, or small cluster of cells will form a visible colony, called a colony-forming unit (CFU), when mixed with the growth medium.[4]
Enumeration of microorganisms requires diluting samples to achieve a population that is countable by the chosen method.
Several techniques have been described and are available for aerobic plate counts: pour plate technique, surface spread plate method, membrane filtration, spiral plate method, calibrated loop method, drop plate method.[4] The choice of the most appropriate method must take into account the requirements of the regulatory authorities, the type of sample to be analysed, the expected microorganisms and level of contamination.
The International Standard ISO 4833-1 specifies a pour plate method for the enumeration of mesophilic organisms and is applicable to products that require a reliable count when a low limit of detection is specified or to products expected to contains spreading colonies.[1]
ISO 4833-2 specifies a surface plating technique applicable to products containing heat sensitive organisms or obligately aerobic bacteria.[2]
ISO 17410 describes a surface plating method for the enumeration of psychrotrophic microorganisms with incubation at 6.5°C.[3]
In USA, detailed procedures for determining the aerobic plate count have been developed by the APHA[4-6], the AOAC[7] and summarised in the FDA Bacteriological Analytical Manual for Foods.[8]
The formulation of Tryptic Glucose Yeast Agar complies with ISO Standards, FDA-BAM and other regulatory authorities. Tryptone provides nitrogen, carbon, minerals and amino acids for the microbial growth. Yeast extract is a source of vitamins, particularly of the B-group. Glucose is a source of carbon and energy.

4A - DIRECTIONS FOR DEHYDRATED MEDIUM
Suspend 23.5 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.

4B - DIRECTIONS FOR READY TO USE FLASKS/TUBES
Liquefy the contents of the flask/tube in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle or the tube 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, mix well and pour the medium 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 Yeast Agar (Plate Count Agar), (Standard Methods Agar	Dehydrated medium	4021452 4021454	500 g (21.3L) 5 Kg (213 L)
Plate Count Agar	Ready to use medium in plates	542145	2 x 10 plates ø 90 mm
Plate Count Agar	Ready to use medium in plates	492145	3 x 10 plates ø 55 mm
Plate Count Agar	Ready to use medium in tubes	552145B	20 x 15 mL
Plate Count Agar	Ready to use medium in flasks	5121452 5121453	6 x 100 mL 6 x 200 mL

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.

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8 – SPECIMENS

Materials of sanitary importance such as products intended for human consumption and the feeding of animals, environmental samples in the area of food production and food handling. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[1-7]

9 - TEST PROCEDURE

Colony count by the pour plate technique.[1]

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 Yeast 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 30 °C for 72 h.

Colony count by the surface plating technique.[2,3]

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 Yeast 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 30 °C for 72 h for the enumeration of mesophilic organisms or at 6.5°C for 10 days for the enumeration of psychrotrophic microorganisms.

Consult the appropriate International Standard for the details of the procedures.[1-7]

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.[1-7]

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	30°C/72H-A	good growth
S. aureus ATCC 6538	30°C/72H-A	good growth
B. subtilis ATCC 6633	30°C/72H-A	good growth

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

12 – PERFORMANCE CHARACTERISTICS

Prior to release for sale, representative samples of all lots of dehydrated and ready to use Tryptic Glucose Yeast Agar are 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 30°C for 72 hours. The colonies are enumerated on both media and the productivity ratio (Pr: CFU_{PCA}/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. 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.[4,9]
- 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. [4,10]
- Increasing the holding time of the dilutions in the diluent leads to higher count. [4,11]
- 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.[4]

14 - PRECAUTIONS AND WARNINGS

- This culture medium is for Laboratory use 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 flasks or tubes 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 or tubes 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 or tubes.
- Once the bottled or tubes medium is liquefied, it cannot be solidified and dissolved a second time.

- Ready-to-use medium in tubes and flasks are sterilised by autoclaving.
- Each ready-to-use plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- 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 plates

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

Ready-to-use medium in flasks and tubes

Upon receipt, store flasks/tubes in their original pack at 2-8°C away from direct light. If properly stored, the flasks/tubes may be used up to the expiration date. Do not use the flasks/tubes beyond this date. Flasks/tubes from opened secondary packages can be used up to the expiration date. Opened flasks/tubes must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use flasks/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 Standards, self-prepared flasks can be stored at +2 °C to +8 °C for up to 3 months and the self-prepared plates can be stored at +2 °C to +8 °C for up to 4 weeks.[1,3]

16 - REFERENCES

1. ISO 4833-1:2013. Microbiology of the food chain – Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30 °C by the pour plate technique.
2. ISO 4833-2:2013. Microbiology of the food chain – Horizontal method for the enumeration of microorganisms - Part 2: Colony count at 30 °C by the surface plating technique.
3. ISO 17410:2019. Microbiology of the food chain — Horizontal method for the enumeration of psychrotrophic microorganisms
4. American Public Health Association. Compendium of Methods for the Microbiological Examination of Foods, 5th ed. 2015. APHA, Washington, DC.
5. American Public Health Association. Standard Methods for the Examination of Water, 23rd ed. 2017. APHA, Washington, DC
6. American Public Health Association. Standard Methods for the Examination of Dairy Products. 17th ed. 2004. APHA, Washington, DC.
7. Association of Official Analytical Chemists. Official Methods of Analysis, 21st ed. 2019. AOAC, Arlington, VA.
8. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 3: Aerobic Plate Count. Rev 2001
9. Berry JM, McNeill DA, Witter LD. Effect of delay in pour plating on bacterial counts. J Dairy Sci 1969; 52:1456-1457
10. Koburger JA. Stack pouring of Petri plates: a potential source of error. J Food Prot. 1980; 43:561-562.
11. Huhtanen CN Brazis AR, Arledge WL et al. Effects of time of holding dilutions on counts of bacteria from raw milk. J Milk Food Technol. 1972; 35:126-130.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT 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 3	Updated layout and content	2022/09

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

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