

OGYE AGAR BASE OXYTETRACYCLINE ANTIMICROBIC SUPPLEMENT GENTAMICIN ANTIMICROBIC SUPPLEMENT

Dehydrated culture medium and selective supplements

1 - INTENDED USE

For the detection and enumeration of yeasts and moulds in foods.

2 – COMPOSITION *

OGYE AGAR BASE

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Yeast Extract	5.0 g
Glucose	20.0 g
Agar	13.0 g

OXYTETRACYCLINE ANTIMICROBIC SUPPLEMENT

(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Oxytetracycline HCl	50 mg
---------------------	-------

GENTAMICIN ANTIMICROBIC SUPPLEMENT

(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Gentamicin	25 mg
------------	-------

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Traditionally, acidified media have been used to enumerate yeasts and moulds and may be appropriate for certain types of foods. However, media supplemented with antibiotics are more commonly used as they may facilitate resuscitation of injured cells.¹

Mossel et al.² in 1962 demonstrated that acidic media were not completely suitable for counting yeasts and moulds in foods for two reasons: 1) heat-stressed yeast cells do not tolerate the acidic conditions necessary to inhibit bacterial contaminants; 2) yeast and mould growth is often limited by the presence of acid-tolerant bacterial flora. Mossel et al.³ described Oxytetracycline-Glucose Yeast Extract (OGYE or OGY) Agar for the selective isolation and enumeration of yeasts and moulds in foods and demonstrated that this medium improved fungal recovery compared to acidified agar media.

Under certain experimental conditions and when testing certain foods, the use of oxytetracycline alone was not sufficient to obtain reliable counts of yeasts and moulds.⁴ Mossel et al.⁵ observed that, with high-protein foods, heavily contaminated with Gram-negative rods, it was necessary to use both oxytetracycline and gentamicin to achieve complete inhibition of contaminants.

OGYE Agar contains yeast extracts which supplies B-complex vitamins to stimulate bacterial growth. Glucose is a source of carbon and energy. Chloramphenicol and gentamicin are inhibitors of a wide range of Gram-negative and Gram-positive bacteria.

4 - DIRECTIONS FOR MEDIUM PREPARATION

A) OXYTETRACYCLINE GLUCOSE YEAST EXTRACT AGAR

Suspend 19 g in 500 mL of cold purified water and heat to boiling to dissolve completely. Autoclave at 115°C for 15 minutes and cool rapidly to approximately 47-50°C. Reconstitute under aseptic conditions one vial of Oxytetracycline Antimicrobial Supplement (code 4240000) with 5 mL of sterile purified water, and add to the base medium. Final concentration of oxytetracycline HCl: 100 mg/L

B) OXYTETRACYCLINE GENTAMICIN GLUCOSE YEAST EXTRACT AGAR

Prepare the medium as described above and add the contents of one vial of Gentamicin Antimicrobial Supplement (code 4240004), reconstituted with 5 mL of sterile purified water. Final concentrations: gentamicin 50 mg/L, oxytetracycline 100 mg/L

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellowish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, clear
Oxytetracycline Antimicrobial Supplement	short, dense, pale-yellow pellet; pale yellow limpid solution after reconstitution
Gentamicin Antimicrobial Supplement	short, dense, white pellet; colourless limpid solution after reconstitution
Final pH at 20-25 °C	6.6 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
OGYE Agar Base	Dehydrated medium	4018382	500 g (13.1 L)
Oxytetracycline Antimicrobial Supplement	Freeze dried supplement	4240000	10 vials, each for 500 mL of medium
Gentamicin Antimicrobial Supplement	Freeze dried supplement	4240004	10 vials, each for 500 mL of medium

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 – SPECIMENS

Food samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.





9 - TEST PROCEDURE

For the enumeration of yeasts and moulds, the following scheme of culture media is recommended:

- OGYE Agar Base + oxytetracycline: general use on food with a low protein content and little contamination with Gram negative bacilli.
- OGYE Agar Base + oxytetracycline and gentamicin: foods with a high protein content in which yeasts and moulds are associated with Gram negative bacilli.

1. Prepare a series of suitable dilutions of the sample.
2. Transfer 1 mL of each sample dilution to empty sterile 9 cm Petri dish (2 plates per dilution).
3. Add approximately 15 mL of melted and cooled medium prepared as described above to each plate. Mix gently turning the plates.
4. Incubate between 22 °C and 25 °C for 5 days, but not longer.

The plates can also be inoculated using surface spreading technique.

10 - READING AND INTERPRETATION

After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies.

Count the colonies in plates containing 50-100 colonies after 5 days or in any countable plates when aerial mycelia seem to obscure further readings after 2 days.

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
<i>Saccharomyces cerevisiae</i> ATCC 9763	25 ± 1°C/ 5 days/A	growth
<i>Aspergillus brasiliensis</i> ATCC 16404	25 ± 1°C/ 5 days/A	growth
<i>Escherichia coli</i> ATCC 25922	25 ± 1°C/ 5 days/A	inhibited
<i>Bacillus subtilis</i> ATCC 6633	25 ± 1°C/ 5 days/A	inhibited

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

12 – PERFORMANCES CHARACTERISTICS

Prior to release for sale, representative samples of all lots of dehydrated OGYE Agar Base supplemented with Oxytetracycline Antimicrobial Supplement (REF 4240000) are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch. Productivity is tested by a quantitative test, with the target strains *S. cerevisiae* ATCC 9763, *C. albicans* ATCC 10231, *A. brasiliensis* ATCC 16404, *P. cyclopium* ATCC 16025; the plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 25° C for 3-5 days in air. 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 and if the colonies morphology and colour are typical the results are considered acceptable and conform to the specifications.

The selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the following strains: *E. coli* ATCC 25922, *B. subtilis* ATCC 6633. The growth of the non-target strain is totally inhibited.

13 – LIMITATIONS OF THE METHOD

- OGYE Agar loses its bacteriostatic properties, especially towards *Bacillus* spp., when medium is heavy inoculated, when incubation is prolonged, or temperature is increased to 35-37°C, or when exposed to proteinaceous substrates.⁶
- Moulds do not grow on OGYE Agar when contamination is low.⁶
- Rapid growth of some moulds may mask colonies of slow growing organisms.⁶
- The medium is not suitable for cultivation of spores of *Byssoschlamys* spp. It is recommended for this genus a chloramphenicol containing medium.⁶
- The spores of moulds disperse in the air with a great facility, handle the Petri dishes with care to avoid development of satellite colonies which would give an overestimation of population in the sample.⁷
- Enumeration methods for yeasts and especially moulds are imprecise because they consist of a mixture of mycelium and asexual and sexual spores. Numbers of colony-forming units depend on the degree of fragmentation of mycelium and the proportion of spores able to grow on the plating medium.⁷
- Non-linearity of counts from dilution plating often occurs, i.e., 10-fold dilutions of samples often do not result in 10-fold reductions in numbers of colonies recovered on plating media. This has been attributed to fragmentation of mycelia and breaking of spore clumps during dilution in addition to competitive inhibition when large numbers of colonies are present on plates.⁷
- It is recommended to perform identification tests on isolates from pure cultures.

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplements are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplements shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Oxytetracycline Antimicrobial Supplement and gentamicin Antimicrobial Supplement Before are classified as hazardous. Consult the Material Safety Data Sheets.
- 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 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.
- Be careful when opening the metal ring of the supplements vials to avoid injury.
- The supplements are sterilized by membrane filtration.



- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets 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

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).

Freeze-dried supplement













Upon receipt, store the product in the original package at +2°C /+8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

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/flasks) and the applied storage conditions (temperature and packaging). According to MacFaddin, OGYE Agar Base without antibiotics may be stored at 2-8°C in the dark for up to 6 months while the medium completed with antibiotics should be used immediately.

16 – REFERENCES

1. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.
2. Mossel DAA, Visser M, Mengerink WHJ. A comparison of media for the enumeration of moulds and yeasts in foods and beverages, Lab Pract 1962; 11:109.
3. Mossel DAA, Kleynen-Semmeling AM, Vincentie HM, Beerens H, Catsaras M. Oxytetracycline-glucose-yeast extract agar for selective enumeration of moulds and yeasts in foods and clinical material. J Appl Bacteriol 1970; 33:454-7.
4. Put HM The limitation of oxytetracycline as a selective agent in media for the enumeration of fungi in soil, feeds and foods in comparison with the selectivity obtained by globenicol (chloramphenicol). Arch. Lebensmittel Hyg. 1974; 25:73.
5. Mossel DAA, Vega CL, Put HM. Further studies on the suitability of various media containing antibacterial antibiotics for the enumeration of moulds in food and food environments J Appl Bacteriol 1975; 39:15-22.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
7. ISO 21527-1:2008. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds - Part 1: Colony count technique in products with water activity greater than 0,95.

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	

REVISION HISTORY

Version	Description of changes	Date
Revision 3	Updated layout and content	2022/11

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

