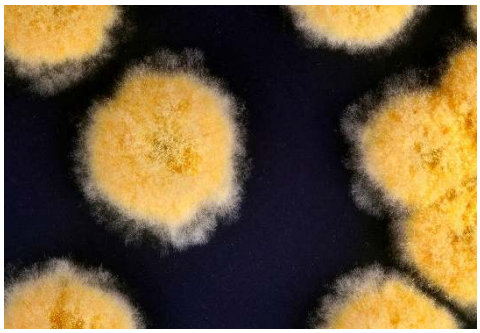
DG18 CHLORAMPHENICOL AGAR

Dehydrated culture medium



DG18 Chloramphenicol Agar colonies of *Eurotium rubrum*

1 - INTENDED USE

Culture medium completed with chloramphenicol for the enumeration of yeasts and moulds in foods and animal feeding stuffs with water activity less than or equal to 0.95 (ISO 21527-2).

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

Enzymatic digest of casein	5 g
D-glucose	10 g
Potassium dihydrogen phosphate	1 g
Magnesium sulphate	0.5 g
Dichloran (2,6-dichloro-4-nitroaniline)	0.002 g
Chloramphenicol	0.1 g
Agar	13.5 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

DG18 (dichloran 18% mass fraction glycerol) Chloramphenicol Agar is a low water activity medium, devised by Hocking and Pitt for enumeration of xerophilic fungi from low-moisture foods.[1] It is recommended by ISO 21527-2 and by FDA-BAM for the enumeration of viable osmophilic yeasts and xerophilic moulds in products intended for human consumption or feeding the animals, having a water activity less than or equal to 0.95, by means of the colony count technique.[2,3]

Glycerol in the medium reduces the water activity from 0.999 to 0.95. Pitt and Hocking[4] showed that glycerol is a suitable solute for the cultivation of a range of xerophilic fungi: it is less inhibitory than NaCl to some species, produces transparent media, and is more readily handled than sugars are at high concentrations. Dichloran (2,6-dichloro-4-nitroaniline) has been shown to inhibit spreading of mucoraceous fungi and to limit colony diameters of other genera in a fungal enumeration medium for foods.[5] The enzymatic digest of casein provides nitrogen, carbon, minerals and amino acids for the microbial growth. Glucose is a source of carbon and energy. Potassium dihydrogen phosphate buffers the medium. Magnesium sulphate enhances the mycological growth. The selective properties of the medium are increased by the presence of chloramphenicol already included in the dehydrated medium: it is a broad-spectrum antibiotic, which is inhibitory to a wide range of Gram-negative and Gram-positive bacteria.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 30.1 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and add 220 g of Glycerol anhydrous (REF 421015). Sterilize by autoclaving at 121°C for 15 minutes. Cool to 44-47°C, mix well and distribute 15 mL amounts into sterile Petri dishes. Avoid exposure of the medium to light.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	beige, clear to slightly opalescent
Final pH at 20-25 °C	5.6 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
DG 18 Chloramphenicol Agar	Dehydrated medium	401394C2	500 g (16.6 L)

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, Glycerol anhydrous (REF 421015), ancillary culture media and reagents.

8 – SPECIMENS

Products intended for human consumption or feeding the animals having a water activity less than or equal to 0.95 such as dry fruits, cakes, jams, dried meat, salted fish, grains, cereals and cereals products, flours, nuts, spices and condiments, etc. Prepare the test sample in accordance with the specific International Standard dealing with the product concerned. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

9 - TEST PROCEDURE

The working procedure described here is taken from ISO 21527-2.[2]

1. On to one DG18 Chloramphenicol Agar plate, using a fresh sterile pipette, transfer 0.1 mL of the test sample if liquid, or 0.1 mL of the initial suspension in the case of other products.
2. Repeat this operation with subsequent dilutions, using a new sterile pipette for each decimal dilution.
3. To facilitate enumeration of low populations of yeasts and moulds, volume up to 0.3 mL of 10^{-1} dilution of sample, or of test sample if liquid, can be spread on to three plates
4. Spread the liquid over the surface of the agar with a sterile spreader, until the liquid is completely absorbed into the medium.
5. Incubate aerobically the inoculated plates in an upright position at 25 ± 1°C for 5 to 7 days.

10 - READING AND INTERPRETATION

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

If necessary, carry out an examination with a binocular magnifier or with a microscope in order to distinguish between cells of yeasts or moulds and bacteria from colonies.

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Read the plates after 2 days, 5 days and 7 days of incubation. If the presence of *Xeromyces bisporus* is suspected, incubate the plates for 10 days.
Select the dishes containing less than 150 colonies/propagules/germs and count these colonies/propagules/germs,
Report as number of colonies/propagules/germs per gram of food.

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.[2]

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
Saccharomyces cerevisiae ATCC 9763	25 ± 1°C/ 5 days/A	growth
Wallemia sebi ATCC 42694	25 ± 1°C/ 5 days/A	growth
Aspergillus restrictus ATCC 42693	25 ± 1°C/ 5 days/A	growth with limited colony spreading
Eurotium rubrum ATCC 42690	25 ± 1°C/ 5 days/A	growth with limited colony spreading
Escherichia coli ATCC 25922	25 ± 1°C/ 5 days/A	inhibited
Bacillus subtilis 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 DG 18 Chloramphenicol Agar are tested for productivity and selectivity by comparing the results with the Reference Batch: Sabouraud Dextrose Agar (productivity).
Productivity is tested by a quantitative test with the target strains *S. cerevisiae* ATCC 9763, *W. sebi* ATCC 42694, *A. restrictus* ATCC 42693, *E. rubrum* ATCC 42690; the plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 25 ± 1°C for 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 ≥ 0.5 and if the colonies morphology and colour are typical and if the colony spreading is limited 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 strains is totally inhibited.

13 – LIMITATIONS OF THE METHOD

- Avoid exposure of the medium to light, since cytotoxic breakdown products can result in underestimation of mycoflora in samples.[2]
- DG 18 Chloramphenicol Agar and the procedure taken from ISO 21527-2 do not apply to dehydrated products with water activity less than or equal to 0,60 and do not allow the enumeration of mould spores.[2]
- 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.[2]
- 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.[2]
- 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.[2]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that identification testing be performed on isolates, from pure culture.

14 - PRECAUTIONS AND WARNINGS

- DG 18 Chloramphenicol Agar 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. Dehydrated DG 18 Chloramphenicol Agar is classified as dangerous. 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 medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the inoculated plates 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 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/flasks) and the applied storage conditions (temperature and packaging). According Baird RM et al. the self-prepared plates can be stored at +2°C +8°C in the dark and protected against evaporation for up to 7 days.[6]

16 – REFERENCES

1. Hocking, A.D.,and Pitt,J.L. (1980) Dichloran-glycerol medium for enumeration of xerophilic fungi from low moisture foods. Appl. Enviornm. Microbiol 39,488-492.
2. ISO 21527-2:2008. 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 less than or equal to 0,95.
3. FDA-BAM Chapter 18: Yeasts, Molds and Mycotoxins. Content current as of: 10/31/2017.
4. Pitt, J. I., and A. D. Hocking. 1977. Influence of solute and hydrogen ion concentration on the water relations of some xerophilic fungi. J. Gen. Microbiol. 101:35-40.
5. King, A. D., A. D. Hocking, and J. I. Pitt. 1979. Dichloran-rose bengal medium for enumeration and isolation of molds from foods. Appl. Environ. Microbiol. 37:959-964.
6. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 216-218.

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	2022/07

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

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