



### Specification

Solid differential and low water activity medium used for the determination of xerophilic fungi in low moisture food and indoor air, according to ISO standard 16000-17:2008.

### Formula \* in g/L

Peptone .....	5.000
Dextrose .....	10.000
Potassium dihydrogen phosphate .....	1.000
Magnesium sulphate heptahydrate .....	0.500
Dichloran .....	0.002
Chloramphenicol .....	0.100
Agar .....	15.000

Final pH 5.6 ±0.2 at 25 °C

\* Adjusted and /or supplemented as required to meet performance criteria

### Directions

Suspend 31,7 g of powder in 1 L of distilled water and bring to the boil. Add 220 g (~180 mL) of glycerol and homogenize. Distribute it into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

### Description

Among the culture media for xerophilic fungi, those that have played a more successful role are the ones which include any agent that restrains the continuous growth of zygomycete fungal colonies. Dichloran (dichlorebenzalkonium chloride) and Rose Bengal are two of those inhibitors.

DG18 Agar formulation used is that proposed by Hocking & Pitt in 1980, and it includes Dichloran which limits the size of fungal colonies more efficiently than Rose Bengal. Chloramphenicol inhibits bacterial growth and its thermostability allows it to be included in the medium before sterilization.

The inclusion of 18% (w/w) of Glycerine gives the medium a water activity ( $a_w$ ) of 0,955 without causing any of the problems that generally occur when this water activity is provided by sodium chloride or sugar.

The addition of Triton X-301® (Tapia de Daza and Beuchat, 1992) at a concentration of 0.01% (w/w) enables easier enumeration of xerophiles when *Eurotium spp.* are present (Beuchat and Hwang).

### Technique

Mass inoculation is recommended by spread plating using an inoculation loop, a swab or by spreading the sample with a Drigalsky loop. Never use an inoculum volume greater than 0.1 ml.

According to the standardized technique, plates must be incubated à 22-25 °C, with partial readings after 3 and 5 days, and definitive readings after 7-8 days. Results are expressed in xerophiles - CFU/g or ml of food samples or CFU/m<sup>3</sup> of air.

Plates of DG 18 Agar in bags will keep for up to one week à 5 ±3 °C in the dark. Due to its extreme water activity ( $a_w$ = 0.955), the plates must be rejected if any kind of dehydration is suspected.

### Quality control

**Incubation temperature:** 25 ± 1 °C

**Incubation time:** 48 h - 5 days

**Inoculum:** Practical range 100 ±20 CFU. min. 50 CFU (productivity)/ 10<sup>4</sup>-10<sup>6</sup> CFU (selectivity), according to ISO 11133:2014/Amd 1:2018.

Microorganism	Growth	Remarks
<i>Bacillus subtilis</i> ATCC® 6633	Inhibited	-
<i>Escherichia coli</i> ATCC® 25922	Inhibited	-
<i>Wallemia sebi</i> ATCC® 42694	Productivity > 0.50	-
<i>Saccharomyces cerevisiae</i> ATCC® 9763	Productivity > 0.50	-
<i>Candida albicans</i> ATCC® 10231	Productivity > 0.50	-
<i>Aspergillus niger</i> ATCC® 16404	Productivity > 0.50	-



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### References

- BEUCHAT, L.R. and C.A. HWANG (1995) Evaluation of modified dichloran 18% glycerol (DG18) agar for enumerating fungi in wheat flour. *Int. J. Food Microbiol.* 29:161-166.
- HOCKING, A.D. and J.I. PITT (1980) Dichloran-glycerol medium for enumeration of xerophilic fungi from low-moisture food. *Appl. Environm. Microbiol.* 39:488-492.
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- ISO 16000-17 Standard. (2008) Indoor air.- Part 17: Detection and enumeration of moulds - Culture-based method.
- ISO 21527-2 :2008. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds- Part 2: Colony count technique in products with water activity less than or equal to 0,95.
- PITT, J.I., and A.D. HOCKING (1985) *Fungi and Food Spoilage*. Academic Press. Sydney.
- PITT, J.I., A.D. HOCKING and D.R: GLENN (1983) An improved medium for the detection of *Apergillus flavus* and *A. parasiticus*. *J. appl. Bacteriol.* 54:109-114.
- SAMSON, R.A., E.S. HOEKSTRA, J.C. FRISVAD and O. FILTENBORG (2002) *Introduction to the Food Borne Fungi*. 6th ed. Centraalbureau voor Schimmelcultures. Utrech.
- TAPIA de DAZA, M.S. and L.R. BEUCHAT. (1992) Suitability of modified dichloran glycerol (DGH!8) agar for enumerating unstressed and stressed xerophilic molds. *Food Microbiol.* 9:319-333.

### Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).