

OGYE AGAR BASE
OXYTETRACYCLINE ANTIMICROBIC SUPPLEMENT
GENTAMICIN ANTIMICROBIC SUPPLEMENT

Basal medium and selective supplements for the enumeration of yeasts and moulds

TYPICAL FORMULAS**OGYE Agar Base - (g/l)**

Yeast Extract	5.0
Glucose	20.0
Agar	13.0

Oxytetracycline Antimicrobial Supplement (vial contents for 500 ml of medium)

Oxytetracycline HCl 50 mg

Gentamicin Antimicrobial Supplement (vial contents for 500 ml of medium)

Gentamicin 25 mg

DIRECTIONS FOR POWDERED MEDIUM**A) OXYTETRACYCLINE GLUCOSE YEAST EXTRACT AGAR**

Suspend 19g of OGYE Agar Base in 500ml of cold distilled water and heat to boiling. Autoclave at 115°C for 15 minutes and cool to approximately 50°C. Reconstitute under aseptic conditions one vial of Oxytetracycline Antimicrobial Supplement (code 4240000) with 5ml of sterile distilled 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 5ml of sterile distilled water. Final concentration: gentamicin 50mg/l, oxytetracycline 100 mg/l

Final pH (after Oxytetracycline Antimicrobial Supplement addition) 6.6 ± 0.2

DIRECTIONS FOR READY TO USE MEDIUM IN FLASKS

Heat to boiling the ready to use medium in flasks and cool to 45-50°C. Reconstitute under aseptic conditions the contents of one vial of Oxytetracycline Antimicrobial Supplement (code 4240000) and the contents of one vial of Gentamicin Antimicrobial Supplement (code 4240004) with 5ml of sterile distilled water. Add 1 ml of the suitable supplement to the base medium: Mix well and distribute into sterile Petri dishes.

Final pH (after Oxytetracycline Antimicrobial Supplement addition) 6.6 ± 0.2

LIMITATION

Oxytetracycline Antimicrobial Supplement is suitable for the supplementation only of Biolife Oxytetracycline Glucose Yeast Extract Agar. Do not use medium base and supplements of different suppliers.

DESCRIPTION

It has been demonstrated by Mossel et al. (1962) that acidic media are not completely suitable for counting yeasts and moulds in foods for two reasons:

- 1) Yeast cells stressed by heat, do not tolerate the acid conditions necessary to inhibit bacterial contaminants.
- 2) Yeast and mould growth is often limited by the presence of acid-tolerant bacterial flora.

The addition of oxytetracycline to a neutral pH medium has proved to be particularly suitable since it provides higher yeast and mould counts than previously used acid pH media. These observations were confirmed by Buttiaux and Catsaras, and by Sainclivier and Roblot, in a study carried on 4000 clinical and food specimens. Mossel et al. (1970), estimated the accuracy of yeast and mould counts using OGYE Agar. Furthermore, they observed a complete inhibition of *Bacillaceae*. Under certain experimental conditions and when testing certain foods, the use of oxytetracycline alone was not sufficient to obtain reliable yeast and mould counts (Put, 1974). In particular, Mossel et al. (1979) observed that, with very proteinaceous foods heavily contaminated with Gram-negative rods, it is

necessary to use both oxytetracycline and gentamicin in order to obtain complete inhibition of contaminants. Moreover, when a higher incubation temperature is required the use of oxytetracycline in proteinaceous food tests has been seen to be less suitable because it is 50% inactivated when incubated for 5 days at 37°C (Mossel, 1970; Put, 1974). To avoid this inconvenience and also when it is necessary to limit the overgrowth of moulds (*Neurospora* and *Rhizopus* spp.), and the production of aerial mycelia, the use of Rose Bengal Agar is advised.

ISO 7954, for the enumeration of yeasts and moulds at 25°C recommends the use of Chloramphenicol Glucose Yeast Extract Agar (cat. N° 401289) or alternatively the use of OGYE Agar Base supplemented with 100mg/l of oxytetracycline HCl.

TECHNIQUE

Prepare a series of suitable dilutions of the sample. Transfer 1ml aliquots to an empty 9cm Petri dish. Add approximately 15ml of medium prepared as described above. Mix gently turning the plates. Incubate for 5 days at 22 ± 2°C checking for the formation of aerial mycelia after 2 days. Count the colonies in plates containing 50-10 colonies after 5 days or in any countable plates when aerial mycelia threaten to obscure further readings after 2 days.

USER QUALITY ASSURANCE (25°C - 3 DAYS)

Productivity control

C.albicans ATCC 10231: good growth

A.niger ATCC 16404: good growth

P.cyclopium ATCC 16025: good growth

S.cerevisiae ATCC 9763: good growth

Selectivity control

E.coli ATCC 25922: inhibited

B.subtilis ATCC 6633: inhibited

STORAGE

Dehydrated medium: 10-30°C

User prepared flasks (medium base): 3 months at 2-8°C

User prepared plates: 7 days at 2-8°C

REFERENCES

- Buttiaux, R. et Catsaras, M. (1965).. Annl, Inst. Pasteur, Lille, **16**,167.
- ICMSF (1978). Microorganisms in Foods, Their Significance and Methods of Enumeration. University of Toronto Press, 157-159.
- ISO 7954:1987 Microbiology-General guidance for enumeration of yeasts and moulds -Colony count technique at 25 degrees C.
- Jarvis, B. (1973). J. App. Bact. 36, 723-727.
- Mossel, D.A.A., Visser, M. and Mengerink, W.H.J. (1962) Lab. Pract. **11**, 109.
- Mossel, D.A.A., Kleyen-Semmeling A.M.C., Vincentie H.M. (1970). J. App. Bact. 33, 454
- Mossel, D.A.A et al. (1979). J. App. Bact. **39**, 15
- Put H.M. (1974) Arch. Lebensmittel Hyg. **25**, 73.
- Sainclivier M. and Roblot A.M. (1966) Annl, Inst. Pasteur, Lille, **17**, 181.

PACKAGING

4018382 OGYE Agar Base

500g (13.1 l)

4018384 OGYE Agar Base

5kg (131 l)

5118382 OGYE Agar Base

6 x 100 ml ready to use flasks

4240004 Gentamicin Antimicrobial Supplement

10 vials, each for 500ml of medium

4240000 Oxytetracycline Antimicrobial Supplement

10 vials, each for 500ml of medium