

Oxford agar – Modified (MC)

This monograph has been assessed by members of the IUMS-ICFMH Working Party on Culture Media and given 'Approved' status.

Description and history

Modified Oxford agar (MC) is similar to Oxford agar (q.v.) developed by Curtis et al. (1989a). In MC the lithium chloride content is reduced to 12 g/l to permit the growth of some of the more sensitive *Listeria monocytogenes* strains (e.g. ATCC 35152 and CDC F4561). Ceftazidime (20 mg/l) replaces the cycloheximide, acriflavine, cefotetan and fosfomycin used in Oxford agar. Limited experience has indicated that *Staphylococcus* spp. and fungal growth from food samples are not a problem with MC. However, the *Listeria monocytogenes* strain ATCC 35152 and some strains of *Listeria seeligeri* and *Listeria ivanovii* are inhibited on MC at 35°C (Curtis et al., 1989b). Thus incubation at 30°C is preferred for the optimal recovery of *Listeria* spp. on this medium. A suitable MC agar should support good growth of *Listeria monocytogenes* strain ATCC 35152 at 30°C and suppress the growth of the more resistant strains of *Enterococcus faecalis* (e.g. FSIS 16a).

Composition (grams)

Columbia agar base (Oxoid CM331)	39.0
Aesculin	1.0
Iron (III) ammonium citrate	0.5
Lithium chloride	12.0
Colistin, methane sulphonate (Sigma) or sulphate	0.01
Ceftazidime pentahydrate (Glaxo)	0.02
Distilled or deionized water	1000.0

Preparation

Suspend all the ingredients except ceftazidime in a bottle or flask containing a magnetic stirring bar and autoclave the medium for 12 min at 121°C. Mix the medium after autoclaving and cool to 46°C. Add 2 ml of filter sterilized 1% ceftazidime solution per litre of the autoclaved, cooled molten base while stirring with a magnetic mixer. Dispense 12 ml per 9 cm diameter Petri dish.

Physical properties

Appearance	Light yellow or tan. Dark colour indicates overheating and decomposition of aesculin.
pH	7.2 ± 0.2

Shelf life

Ready to use plates 14 days at $4 \pm 2^\circ\text{C}$.

Inoculation methods for samples

1. From enrichment broths, soak a sterile cotton swab in the enrichment broth and swab it on half of an MC agar plate, then streak for isolation with a sterile loop in two 90° directions.
2. For direct plating and counting of $>100/\text{g}$ of *Listeria* spp. in foods, make decimal dilutions of the foods and spread plate 0.1 ml of each dilution on to MC agar.
3. For the rapid detection of $>100/\text{g}$ of *Listeria* spp. growing in many kinds of refrigerated foods, wipe a cotton swab moistened with sterile pH 7.2 PBS or similar phosphate buffer over the interface of vacuum packaged refrigerated foods or just under the surface of soft ripened cheeses. Swabs may also be dipped into food homogenates. MC agar plates should then be inoculated by the swab and streak procedure described above. Isolated listeria-like colonies on the swab and streak plate can be recognised and picked for further identification. The number of listeria colonies on the swab and streak MC plates can be estimated by comparing the density of colonies on the inoculated area with that of standard inocula in a similar manner to the Dip Slide procedure of Guttman and Naylor (1967).

Incubation method

At 30°C for 26 h and 40 h, in air.

Reading of results and interpretation

Typical *Listeria* spp. form distinctive small (1 mm) white hemi-spherical colonies after 26 h incubation at 30°C which can be recognized easily with some practice. It is best to ignore the blackening or black zones on MC agar entirely when working with mixed cultures as this can be caused by other bacteria and become confusing. In limited observations, no additional *Listeria monocytogenes* colonies were recovered from MC agar after 26 h incubation. The 40 h incubation is used to detect other, slow growing, *Listeria* spp. Additional confirmatory tests are necessary to identify various *Listeria* species.

Quality assessment(i) *Productivity*

Test strains	<i>Listeria ivanovii</i> serovar 5 NCIMB 50095 <i>Listeria monocytogenes</i> serovar 1/2a (ATCC 35152/ NCTC 7973) <i>Listeria monocytogenes</i> serovar 4b (ATCC 13932/ NCTC 10527)
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Supplementary strain	<i>Listeria monocytogenes</i> sv 4a (ATCC 19114/NCTC 5124)
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Inoculation method	Modified Miles-Misra or spiral plating.
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(ii) *Selectivity*

Test strains	<i>Enterococcus faecalis</i> NCIMB 50030 <i>Proteus mirabilis</i> (ATCC 29906/CECT 4168/NCTC 11938)
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Supplementary strain	<i>Staphylococcus aureus</i> NCIMB 50080
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Inoculation method	Modified Miles-Misra or spiral plating.
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(iii) *Characteristic appearance of colonies*

See above.

References

- Curtis, G.D.W., Mitchell, R.G., King, A.F. and Griffin, E.J. (1989a) A selective differential medium for the isolation of *Listeria monocytogenes*. Lett. Appl. Microbiol. 8, 95–98.
- Curtis, G.D.W., Nichols, W.W. and Falla, T.J. (1989b) Selective agents for listeria can inhibit their growth. Lett. Appl. Microbiol. 8, 169–172.
- Guttmann, D. and Naylor, G.R.E. (1967) Dip Slide: an aid to quantitative urine culture in general practice. Br.Med. J. 3, 343–345.