Gram Positive Cocci

X012

COLISTIN, NALIDIXIC ACID for the preparation of Columbia C.N.A. medium.

A medium selective for Gram positive cocci is obtained when this antibiotic mixture is added to LAB001 Columbia Agar.

Final Concentration	**mg/litre**
Colistin	10
Nalidixic acid	10
Add 1 vial X012 to 500ml medium	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, together with any other additives, mix gently and pour.

Reference:

Ellner, P.D., Stossel, C.I., Drakeford, E., Vasi, F. (1966). "A new culture medium for medical bacteriology." Amer. J. Clin. Path. 45: 502.

Haemophilus influenzae

X260

BACITRACIN for the isolation of *Haemophilus influenzae.*

Suitable for use with Columbia blood agar base and other blood agars supplemented with heated ("chocolated") blood.

Final Concentration	mg/litre
Bacitracin	75
Add 1 vial of X260 to 1 litre of medium.	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium with heated blood cooled to 47°C, mix well and pour.

Helicobacter pylori

X040

VANCOMYCIN, CEFSULODIN, AMPHOTERICIN, for the isolation of *Helicobacter pylori.*

For addition to *Helicobacter pylori* medium LAB 140

Final Concentration	mg/litre
Cefsulodin	10
Vancomycin	10
Amphotericin	20
Add 1 vial to 500ml medium	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, along with other additives, mix well and pour.

Impedance Microbiology

X137

T.M.A.O. Selenite for inclusion in Easter and Gibson Salmonella Detection Medium LAB 137.

The growth of *Salmonella* in the medium reduces T.M.A.O. to T.M.A. and in so doing, significantly increases the conductivity of the medium. The incorporation of sodium biselenite makes the medium selective for salmonellae.

Final Concentration	g/litre
T.M.A.O. (Trimethylamine-N-oxide)	5.0
Sodium biselenite	4.0
Add 1 vial X137 to 100ml medium	

Reconstitute contents with 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C. Swirl to mix then dispense into sterile containers.

References:

Easter, M.C., Gibson, D.M. (1985). Rapid and automated detection of *Salmonella* by electrical measurements, J. Hyg. 94: 245-262.

Gibson, D.M. (1987). Some modifications to the media for rapid automated detection of salmonellas by conductance. H. Appl. Bacteriol. 63: 299-304.

Odgen, I.D., Cann, D.C. (1987). Amodified conductance medium for the detection of *Salmonella* spp. J. Appl. Bacteriol. 63: 359-464.

Listeria

X122

C.C.C.A.F. CEFOTETAN, CYCLOHEXIMIDE, COLISTIN, ACRIFLAVINE, FOSFOMYCIN, for the isolation of *Listeria monocytogenes* from environmental, clinical and food samples.

For addition to LAB 122 Listeria Isolation Medium or HAL 2 Harlequin™ Listeria Medium.

Final Concentration	mg/litre
Cefotetan	2
Cycloheximide	400
Colistin	20
Fosfomycin	10
Acriflavine	5
Add 1 vial of X122 to 500ml of LAB 122.	
Add 1 vial of X122 to 1 litre of HAL 2.	

Reconstitute contents of vial by the addition of sterile 50% ethanol in water. Add aseptically to sterilised medium cooled to 47°C, mix gently then pour.

Reference:
Curtis, *et al.* (1989). A selective differential medium for the isolation of *Listeria monocytogenes.* Lett. in Appl. Microbiol. 8: 95-98.

X138

N.A.C. NALIDIXIC ACID, ACRIFLAVINE, CYCLOHEXIMIDE for the selective enrichment broth culture of *Listeria monocytogenes.*

For addition to LAB 138 Listeria Enrichment Broth recommended by the F.D.A. for Listeria isolation from food and environmental samples and LAB 139 Buffered Listeria Enrichment Broth.

Final Concentration	mg/litre
Nalidixic acid	40
Cycloheximide	50
Acriflavine	15
Add 1 vial of X138 to 500ml medium	

Reconstitute contents of vial by the addition of sterile 50% ethanol in water. Add aseptically to sterilised medium cooled to 47°C, mix gently then pour.

Reference:
Lovett et al. (1987). *Listeria monocytogenes* in raw milk: detection incidence and pathogenicity. J. Food Protect. 50: 188-192.

X144

P.A.C. supplement for the enrichment and isolation of *Listeria* spp from food and environmental samples.

For the addition to LAB 144 Palcam Broth and Lab 148 Palcam Agar

Final concentration	mg/litre
Polymyxin	10
Acriflavine	5
Ceftazidime	20
Add 1 vial of X144 to 500ml of Palcam Broth or Agar	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, along with other additives, mix well and pour.

X164, X564

1/2 FRASER supplement for the primary enrichment of *Listeria* spp from food and environmental samples.

For addition to LAB 164 Fraser Broth Base

Final Concentration	mg/litre
Ferric ammonium citrate	500
Acriflavine	12.5
Nalidixic acid	10
Add 1 vial of X164 to 450ml of Fraser Broth Base	
Add 1 vial of X564 to 2.25 litres of Fraser Broth Base	

Rehydrate contents of vial with 2ml 50% methanol (5ml for X564). Add aseptically to sterilised medium cooled to 47°C, mix well and pour.

X072

POLYMYXIN B, CEFTAZIDIME supplement for the isolation of *Listeria monocytogenes*.

For addition to LAB 172, LMBA

Final Concentration	mg/litre
Polymyxin B	10
Ceftazidime	20
Add 1 vial X072 and 1 vial of X072N to 500ml medium.	

Rehydrate contents of vial by the addition of 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and pour.

X072N

NALIDIXIC ACID supplement for the isolation of *Listeria monocytogenes.*

For addition to LAB 172, LMBA

Final Concentration	mg/litre
Nalidixic acid	40
Add 1 vial X072N and 1 vial of X072 to 500ml medium.	

Rehydrate contents of vial by the addition of 5 ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and pour.

X165

FRASER supplement for the secondary enrichment of *Listeria* spp from food and environmental samples.

For addition to LAB 164 Fraser Broth Base

Final Concentration	mg/litre
Ferric ammonium citrate	500
Acriflavine	25
Nalidixic acid	20
Add 1 vial of X165 to 500ml of Fraser Broth Base	

Rehydrate contents of vial with 2ml 50% methanol. Add aseptically to sterilised medium cooled to 47°C, mix well and pour.

X155, X555

UVMI. Supplement for the primary enrichment of *Listeria* spp from food and environmental samples.

For addition to LAB 155 UVM Broth Base

Final Concentration	mg/litre
Nalidixic acid	20
Acriflavine	12
Add 1 vial of X155 to 500ml of UVM Broth Base	
Add 1 vial of X555 to 2.25 litres of UVM Broth Base	

Rehydrate contents of vial with 5ml sterile deionised water (10ml for X555). Add aseptically to sterilised medium cooled to 47°C, mix well and pour.

X156

UVMII. Supplement for the secondary enrichment of *Listeria* spp from food and environmental samples.

For the addition to LAB 155 UVM Broth Base

Final Concentration	mg/litre
Nalidixic acid	20
Acriflavine	25
Add 1 vial of X156 to 500ml of UVM Broth Base	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix well and pour.

X123

C.N.C.A.F. CEFOTETAN, NATAMYCIN, COLISTIN, ACRIFLAVINE, FOSFOMYCIN.

An alternative natamycin supplement for the isolation of *Listeria* spp. from environmental, clinical and food samples. For addition to LAB 122 *Listeria* Isolation Medium.

Final Concentration	mg/litre
Cefotetan	2
Natamycin	25
Colistin	20
Fosfomycin	10
Acriflavine	5
Add 1 vial X123 to 500ml medium	

Rehydrate the contents of vial by the addition of 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and pour.

X139, X539

N.A.N. NALIDIXIC ACID, ACRIFLAVINE, NATAMYCIN.

An alternative natamycin based supplement for the selective enrichment broth culture of *Listeria* spp. For addition to LAB 138, *Listeria* Enrichment Broth and LAB139, Buffered *Listeria* Enrichment Broth.

Final Concentration	mg/litre
Nalidixic acid	40
Acriflavine	15
Natamycin	25
Add 1 vial of X139 to 500ml medium.	
Add 1 vial of X539 to 2.25 L medium.	

Rehydrate contents of vial by the addition of 5ml of sterile deionised water (10ml for X539). Add aseptically to sterilised medium cooled to 47°C, mix gently and dispense.

Mycobacterium tuberculosis

X124

P.T.T.A. POLYMYXIN B, TICARCILLIN, TRIMETHOPRIM, AMPHOTERICIN supplement for the isolation of *Mycobacterium tuberculosis* from clinical samples.

For addition to LAB 123 Kirchner's T.B. Medium.

Final Concentration	mg/litre
Polymyxin B	200,000 I.U.
Ticarcillin	100
Trimethoprim	10
Amphotericin	10
Add 1 vial of X124 to 500ml medium.	

Rehydrate contents of vial by the addition of 5 ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and dispense.

Neisseria gonorrhoeae

X070, X270

L.C.A.T. LINCOMYCIN, COLISTIN, AMPHOTERICIN, TRIMETHOPRIM for the isolation of *Neisseria* spp. from clinical material.

L.C.A.T. is often preferred to X068 V.C.N.T. for the isolation of *N. gonorrhoeae* because of the emergence of vancomycin sensitive strains. The antifungal agent amphotericin is more readily soluble andtherefore a more active antifungal than nystatin. L.C.A.T. is quoted as the selective agent for New York City G.C. agar but can readily be substituted for V.C.N. or V.C.N.T. in Thayer Martin G.C. agar.

Final Concentration	mg/litre
Lincomycin	1
Colistin	6
Amphotericin	1
Trimethoprim	6.5
Add 1 vial X070 to 500ml medium	
Add 1 vial X270 to 1 litre medium	

Rehydrate contents of vial with 5ml sterile 25% alcohol in water. Add aseptically to sterilised medium cooled to 47°C together with other additives, mix gently and pour.

Reference:

Young, H. (1978). Cultural Diagnosis of Gonorrhoea with modified N.Y.C. Medium. Brit. Journ. Ven. Dis. 54: 36-40.

X069, X269

L.C.T. LINCOMYCIN, COLISTIN, TRIMETHOPRIM. A variant of L.C.A.T. with the amphotericin omitted to permit the growth of yeasts.

Concentrations and rehydration as L.C.A.T.
Add 1 vial X069 to 500ml medium
Add 1 vial X269 to 1 litre medium

X068, X268

V.C.N.T. VANCOMYCIN, COLISTIN, NYSTATIN, TRIMETHOPRIM for Thayer Martin Medium.

The addition of trimethoprim in V.C.N.T. inhibits the swarming of *Proteus* spp. which occasionally make interpretation difficult.

Final Concentration	mg/litre
Vancomycin	3
Colistin	7.5
Nystatin	12.5
Trimethoprim	5
Add 1 vial X068 to 500ml medium	
Add 1 vial X268 to 1 litre medium	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47°C together with other additives, mix gently and pour.

Reference:
Thayer, J.D. and Martin, J.E. (1966). Improved medium selective for the cultivation of *N. gonorrhoeae* and *N. meningitidis.* Public Health rep. 81: 559-562.

X271

GROWTH SUPPLEMENT, to improve the isolation of *Neisseria* spp. from selective media.

For addition to GC agar base LAB 67.

Final Concentration	mg/litre
L-cystine	11
L-cysteine	259
Thiamine HCl	0.03
Ferric nitrate	0.2
Co-Carboxylase	1
NAD	1.0
Guanine HCl	0.3
Adenine	10
L-glutamine	100
PABA	0.13
Vitamin B12	0.1
Add 1 vial to 1 litre of medium	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, along with other additives, mix well and pour.

Pseudomonas species

X108

MODIFIED C.F.C. – CEPHALOTHIN, FUCIDIN, CETRIMIDE for the selective isolation of *Pseudomonas* spp.

When added to LAB 108 Pseudomonas Agar, to prepare C.F.C. medium this supplement can be used to select pseudomonads from food and environmental samples.

Final Concentration	mg/litre
Cephalothin	50
Fucidin	10
Cetrimide	10
Add 1 vial X108 to 500ml medium	

Rehydrate contents of vial with 5ml of sterile 50% alcohol. Add aseptically to sterilised medium cooled to 47°C, mix gently and pour.

Reference:
Mead, G.C. and Adams, B.W. (1977). Br. Poult. Sci. 18: 661-667

X107

C.N. CETRIMIDE, NALIDIXIC ACID for the isolation of *Pseudomonas aeruginosa.*

Suitable for use with LAB 108 Pseudomonas Agar to make the medium selective for *Ps. aeruginosa.*

Final Concentration	mg/litre
Cetrimide	200
Nalidixic acid	15
Add 1 vial X107 to 500ml medium	

Rehydrate contents of vial with 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and pour.

Reference: Goto, S., Enomoto, S. 1970. Jap. J. Microbiol. 14: 65-72.

X140

TICARCILLIN, POLYMYXIN, for the isolation of *Burkholderia (Pseudomonas) cepacia*

Suitable for use with LAB 108 pseudomonas selective agar, or specific selective bases such as that described by Gilligan *et al.*

Final Concentration m	g/litre
Ticarcillin	100
Polymyxin	300,000 iu/litre
Add 1 vial to 500ml of medium	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix well and pour.

Reference:
Gilligan, P.H., Gage, P.A., Bradshaw, L.M., Schidlow, D.V., DeCicco, B.T. (1985) Isolation medium for the recovery of *Pseudomonas cepacia* from respiratory secretions of patients with cystic fibrosis. J.Clin.Microbiol. 22 (1) 5-8.

Pre-Incubation Test (P-INC)

X019, X219

PENICILLIN, NISIN, CRYSTAL VIOLET, for accelerated shelf life determination of dairy products.

The Pre-incubation test uses a selective mixture to inhibit Gram positive organisms whilst allowing the growth of Gram negative bacteria, the main cause of post-pasteurisation contamination and a major factor in determining the shelf life of the product. The technique is also useful for monitoring plant hygiene.

Final Concentration	mg/litre
Penicillin	20,000iu/litre
Nisin	40,000iu/litre
Crystal violet	2.0
Add 1 vial of X019 to 200ml of Milk Agar LAB019	
Add 1 vial of X219 to 1 litre of Milk Agar LAB019	

Rehydrate contents of 1 vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix thoroughly and pour plates.

Method A
Pre-incubate test material at 21°C for 24hr. Prepare suitable dilution series, and inoculate Milk Agar plates containing P-INC supplement. Incubate at 21°C for 24hr, and count all colonies (some may be small, use of a hand lens is recommended). Calculate the CFU/ml and using the tables of Griffith's *et al* the shelf life can be determined.

Method B
Rehydrate X219 with 1ml of deionised water only, add 0.1ml to the test material and incubate at 20°C for 24hr. Prepare suitable dilution series, and inoculate Milk Agar plates. Proceed as for Method Aabove.

References:

Griffiths, M.W., and Phillips, J.D. (1985) J.Appl.Bact. 57, 107.

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1980) J. Soc. Dairy Technol. 33, 8.

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1981) J. Soc. Dairy Technol. 34, 142.

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1984) J. Soc. Dairy Technol. 37, 22.

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1984) Rapid detection of post-pasteurised contamination. Hannah Research Inst. Bulletin No.10.

Griffiths, M.W., and Phillips ,J.D., and Muir, D.D. (1984) Dairy Ind. Int. 50 (3) 25

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1984) Postpasteurisation contamination - the major cause of failure of fresh dairy products. Hannah Research Inst.

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1986) Aust. J. Dairy Technol. 41, 77-79.

Salmonella

X150

NOVOBIOCIN, for the isolation of *Salmonella* using semi-solid technology.

For addition to LAB 150 MSRV and LAB 537 Diassalm

Final Concentration	mg/litre
Novobiocin	20 (MSRV)
Novobiocin	10 (Diassalm)
Add 1 vial to 500ml (MSRV)	
Add 1 vial to 1 litre (Diassalm)	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix well and pour.

References:

De Smedt, J.M., and Bolderdijk, R.F., (1986) Dynamics of salmonella isolation with modified semi-solid Rappaport Vassiliadis medium. J.Food Protection 50 658-661

Van Netten, P., Van Der Zee H., and Van Der Moosdijk A., (1991) The use of diagnostic selective semi-solid medium for the isolation of Salsmonella enteritidis from poultry. Proceedings of the 10th symposium on the quality of poultry meat. Spelderholt Beckbergen 56-67.

Staphylococci

X085

EGG YOLK TELLURITE

A sterile emulsion of egg yolk and potassium tellurite for use as a selective and differential agent in Baird-Parker Medium Base LAB 85. The complete medium is selective for *S.aureus,* and the addition of egg yolk tellurite aids differentiation of this organism from others capable of growing on the agar. Presented in 100ml bottles with a tellurite concentration of 0.2% to give a final concentration in the complete medium of 0.01% (w/v). Add 50ml to 1 litre of Baird-Parker Medium Base.

X086

RPF: BOVINE FIBRINOGEN, RABBIT PLASMA, TRYPSIN INHIBITOR, POTASSIUM TELLURITE supplement for the isolation of *Staphylococcus aureus.*

For addition to LAB 85 Baird-Parker Medium.

Final Concentration	**mg/litre**
Bovine Fibrinogen	0.375
Rabbit Plasma	2.5ml
Trypsin Inhibitor	2.5
Potassium Tellurite	2.5
Add 1 vial of X086 to 90ml medium.	

Rehydrate contents of vial by the addition of 10ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and pour.

X207

METHICILLIN, for the isolation of Methicillin Resistant *S.aureus* (MRSA)

Suitable for use with LAB 7 Mannitol salt agar.

Final Concentration	**mg/litre**
Methicillin	4
Add 1 vial of X207 to 1 litre of medium	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix well and pour.

X192

OXACILLIN, POLYMYXIN B supplement for the isolation of Methicillin Resistant *Staphylococcus aureus* (MRSA).

For addition to LAB 192, ORSIM (Oxcacillin Resistant *Staphylococcus* Isolation Medium).

Final Concentration	**mg/litre**
Oxacillin	2
Polymyxin B	25,000 I.U
Add 1 vial of X192 to 500ml medium.	

Rehydrate contents of vial by the addition of 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and dispense.

Streptococci

X013

COLISTIN, OXOLINIC ACID for the selective isolation of streptococci from clinical material.

When added to LAB 1 Columbia agar or LAB 15 Blood Agar Base No. 2, X013 renders the medium selective for streptococci. Alteration in haemolysis patterns may occur when azide or crystal violet are employed as selective agents but this does not occur with X013.

Final Concentration	mg/litre
Colistin	10
Oxolinic acid	5
Add 1 vial X013 to 500ml medium	

Rehydrate contents of vial with 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C together with other additives, mix gently and pour.

Reference:
Petts, D. (1984). Colistin - Oxolinic Acid - Blood Agar: a new selective medium for streptococci. J. Clin. Microbiol. 19: 4-7.

Yeasts and Moulds

X009, X209

CHLORAMPHENICOL for the selective isolation of yeasts and moulds from food, environmental and clinical specimens.

Chloramphenicol's broad antibiotic spectrum suppresses most contaminating bacteria allowing the yeasts and moulds to grow. It can be added to such media as LAB 9 Sabouraud Dextrose Agar, LAB 36 Rose Bengal Chloramphenicol Agar, LAB 37 Malt Extract Agar and LAB 117 Dermatophyte Test Medium to increase their selectivity whilst not lowering the pH. Reduction of pH will increase the selectivity of a yeast and mould medium but will also inhibit some yeasts as well as having a deleterious effect on the agar gel.

Final Concentration	mg/litre
Chloramphenicol	100
Add 1 vial X009 to 500ml medium	
Add 1 vial X209 to 1 litre medium	

Rehydrate contents of vial with 5ml of Ethyl or Methyl alcohol. Add aseptically to sterilised medium cooled to 47°C, mix gently and pour.

References:
Jervis, B. (1973). Rose Bengal Chlortetracycline agar with other media for the selective isolation and enumeration of moulds and yeasts in foods. J. Appl. Bact. 36 Pages 723-727.

X089

OXYTETRACYCLINE for O.G.Y.E. medium.

For use with LAB 89 Oxytetracycline Glucose Yeast Extract Agar for the enumeration of yeasts and moulds from foodstuffs. Highly proteinaceous foods and incubation above 30°C will inactivate oxytetracycline.

Final Concentration	mg/litre
Oxytetracycline	100
Add 1 vial X089 to 500ml medium	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and pour.

References:
Mossel, D.A.A., *et al*. (1970). O.G.Y.E. for the selective enumeration of moulds and yeasts in food and clinical material. J. Appl. Bact. 35: 454-457.

Yersinia

X120

C.I.N. - CEFSULODIN, IRGASAN, NOVOBIOCIN for the isolation of *Yersinia* spp. from clinical and environmental material.

For addition to LAB 120 Yersinia C.I.N. Agar Base used in the selective isolation of *Y. enterocolitica.*

Final Concentration	mg/litre
Cefsulodin	15
Irgasan	4
Novobiocin	2.5
Add 1 vial X120 to 500ml medium	

Rehydrate contents of vial with 5ml of 30% sterile alcohol. Add aseptically to sterilised medium cooled to 47°C, mix gently and pour.

References:

Schiemann, D.A. (1979). Synthesis of a selective medium of Yersinia enterocolitica. Can. J. Microbiol. 25 (2): 1298.

Schiemann, D.A. (1980). Isolation of toxigenic Yersinia enterocolitica from retail pork products. J. Food Prot. 43: 360.

Schiemann, D.A. (1982). Development of a two-step enrichment procedure for recovery of Yersinia enterocolitica from food. Appl. Microbiol. 43 (1): 14.