Fluorescence Agar

A modification of King's medium, formulated for the demonstration of the fluorescein pigment produced by many strains of *Pseudomonas*.

Code: KM8016

Formulation	**(g/l)**
Peptone mixture	20.0
Dipotassium phosphate	1.5
Magnesium sulphate	1.5
Agar	12.0

pH: 7.2 ± 0.2

Directions:
Suspend 35gms of powder, disperse in 1 litre of deionised water containing 10ml Glycerin. Allow to stand for 10 minutes, swirl to mix then sterilise for 15 minutes at 121ºC. Mix well before pouring. Slant over a generous butt if required.

Q.C. organisms: *Ps. aeruginosa*

Storage: Plates up to 7 days at 2-8ºC in the dark.

Inoculation: Surface spreading.

Incubation: 30-37ºC for 24 and 48 hours aerobically.

References
King, E. O., Ward, M. K. and Raney, D. E. 1954. 2 simple media for demonstration of pyocyanin and fluorescein, J. Lab. Clin Med., 44: 301-307.

FRASER BROTH BASE

A liquid media and supplement for the first and second step *Listeria* spp. enrichment and for the enumeration of *Listeria* spp. by MPN method.

Code: KM1033

Typical formula	**(g/l)**
Proteose Peptone	5.00
Tryptone	5.00
Beef Extract	5.00
Yeast Extract	5.00
Sodium Chloride	20.0
Disodium Phosphate anhydrous	9.50
Monopotassium Phosphate	1.35
Aesculin	1.00
Lithium Chloride	3.00
Acriflavin HCl	0.02
Nalidixic Acid	0.02

pH 7.2 +/- 0.2

Directions
Suspend 27.4g of Listeria Fraser Broth Base or 27.4g 500ml of cold distilled water. Heat to boiling until complete dissolution. Autoclave at 121°C for 15 minutes. Cools to room temperature and to each medium add supplement as required and reconstituted with 5ml of cold distilled water. Mix well and pour into sterile tubes or flasks under aseptic conditions. WARNING**:** The media contain acriflavine, a possible mutagen. Do not inhale. In case of eye or skin contact wash affected area thoroughly with soap and water.

Description
Listeria Fraser Broth Base prepared according to the formulation described by Fraser and by ISO 11290-1, a modification of UVM2 Enrichment Broth with the addition of ferric ammonium citrate.

Fraser Broth is recommended by USDA-FSIS for the secondary enrichment of *Listeria* in foodstuff and environmental samples. The presence of *Listeria* in both broths leads the blackening of the culture, due to the reaction of aesculetin, produced by the aesculin hydrolysis, with iron ions.

Method
Primary and secondary enrichment with plating into PALCAM or Oxford plates:
Inoculate 25g of sample, under aseptic conditions, into 225ml of Listeria Fraser Broth Half Concentration. Incubate at 30°C for 18-24 hours. Transfer 0.1ml to 10ml of Listeria Fraser Broth. Incubate at 37°C for 18-24 hours. Extend incubation till 48 hours. Streak onto “Oxford” or “Palcam” media starting from:
primary enrichment tubes
secondary enrichment tubes incubated 18-24 hours
secondary enrichment tubes incubated 48 hours

Pick at least 5 typical colonies form each “Oxford” or “Palcam” plates and inoculate Tryptone Soy Agar plates. Carry on the identification of *Listeria* spp. and *Listeria monocytogenes* by usual biochemical and serological test methods.

Quality assurance (37°C-24hrs)

Productivity control
L.monocytogenes ATCC 19117: growth and blackening

Selectivity control
S.aureus ATCC 25923: inhibited

Storage
Dehydrated medium: 15-30°C
User prepared tubes: 7days at 2-8°C

References
Fraser, J.A., Sperber, W.H. (1988) J. Food Protect. 51, 10, 762-765.
ISO 11290-1 Microbiology of food and animal feeding stuffs-Horizontal method for the detection and enumeration of *Listeria monocytogenes* Part 1 Detection method.

FERMENTATION BROTH BASE

A liquid base medium to be supplemented with carbohydrates for fermentation tests.

Code: KM8882

Typical formula	**(g/l)**
Peptone	10.00
Beef Extract	1.00
Sodium Chloride	5.00
Bromocresol Purple	0.02

pH 6.8 +/- 0.2

Directions
Suspend 16g in 1000ml of cold distilled water. Heat to dissolve, distribute 4.5ml into tubes and sterilise by autoclaving at 121°C 15 minutes. Prepare a 5% carbohydrate solution, sterilised by filtration, and add 0.5ml to the autoclaved tubes.

Description
Fermentation Broth Base, supplemented with the appropriate carbohydrates is used to determine the fermentation reactions of microorganisms, especially *Listeria* spp. It is recommended by ISO 11290 1-2 for the "carbohydrate tests" for the identification of *Listeria monocytogenes*. See the ISO norms for details of the procedures for rhamnose, xylose and/or methyl-α-D-mannopyranoside.

References
ISO 11290-1 (1996) ISO 11290-1–2 (1998) -Microbiology of food and animal feeding stuffs-Horizontal method for detection and enumeration *of L.monocytogenes*- part 1: detection method; part 2: enumeration method.
Manuel Suisse des Denrées Alimentaires, Chapitre 56, Microbiologie, juillet 2000

Fluid Thioglycollate Medium (U.S.P.)

For the isolation of most fastidious organisms as a blood culture medium or for sterility testing.

Code: KM1032

Typical formula	**(g/l)**
Tryptone	15.0
L-Cystine	0.5
Glucose	5.5
Yeast Extract	5.0
Sodium chloride	2.5
Sodium thioglycollate	0.5
Resazurin	0.001
Agar	0.75

pH: 7.1 ± 0.1

Directions
Weigh 29.75 grams, disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix, then bring to the boil to dissolve and dispense 15ml into 6mm x 150mm tubes. Sterilise by autoclaving for 15 minutes at 121ºC. Store at ambient temperature in the dark, but not in the refrigerator. If more than 30% of the medium turns pink (oxidised) the Eh may be restored (once only) by heating in a boiling water bath or by free-steaming.

Description
A medium for sterility tests, prepared according to the specification of the United States Pharmacopeia. Aerobic and anaerobic organisms grow well in this medium even from small inocula. In appropriate tubes or bottles the thioglycollate ensures adequate anaerobic conditions. The low level of agar reduces oxygen diffusion into the medium. The thioglycollate will also serve to inactivate any mercurial compounds used as preservatives.

Appearance: Pale straw colour, clear. Surface may be pink/blue due to oxidation of Resazurin.

Quality assurance organisms: *C. sporogenes, S. aureus*

Storage: Capped container up to 3 months at 15-20ºC in the dark.

Incubation: 30-35ºC aerobically for 14 days.

Growth indicators: Turbidity, colonies in medium.

References
The Pharmacopeia of the United States of America. 21st End. 1985.