LISTERIA (OXFORD) ISOLATION AGAR

A base medium for the detection of *Listeria monocytogenes*

Code: KM1049

Typical formula	**(g/l)**
Columbia Agar Base	41.0
Lithium Chloride	15.0
Aesculin	1.0
Ferric ammonium citrate	0.5

Supplement as required with:**(mg/vial)**	
Cycloheximide	200.0
Colistin sulphate	10.0
Cefotetan	1.0
Acriflavine	2.5
Fosfomicine	5.0

pH 7.0 +/- 0.1

Directions
Suspend 28.7g of Listeria Isolation (Oxford) Agar in 500ml of cold distilled water. Heat to boiling with agitation and autoclave at 121°C for 15 minutes. Cool to 50°C and add the contents of one vial of supplement reconstituted with 5ml of a solution of 1:1 ethanol sterile distilled water solution. Mix well and distribute into sterile petri dishes.

Description
Listeria Isolation (Oxford) Agar is a base medium without antibiotics and without acriflavin, to be used with Listeria supplement for the isolation of *Listeria* spp.
The complete medium is prepared according to Curtis' formulation and is suggested by FIL-IDF and ISO 12560 for the detection of *L. monocytogenes* in milk and dairy products or in other foodstuffs. The complete Oxford medium contains, as inhibitory agents, lithium chloride, active against streptococci, cycloheximide active against yeasts and moulds, cephotetan and phosphomicin active on Gram-positive and Gram-negative bacteria. The medium contains besides ferric-ammonium citrate and aesculin, which is reduced to aesculetin by Listeria spp. with the formation of grey-brown colonies with brown halo.

Method
Streak a loopful of the suitable enriched broth incubated according the directions onto the surface of an Oxford Medium plate to obtain well-isolated colonies. Incubate at 37°C for 48 hours. Observe for the presence of *Listeria* typical colonies: grey-brown colonies with brown or black halo. From each plate select at least 3 colonies and streak onto TSA Yeast Extract plates. With this growth carry out the identification tests.

Quality assurance (37°C – 48hrs)

Productivity control
L.monocytogenes ATCC 19111: growth, grey colonies with black halo
L.monocytogenes ATCC 13932*:* growth, grey colonies with black halo

Selectivity control
E.coli ATCC 25922: inhibited
E.faecalis ATCC 19433: inhibited
C.albicans ATCC 10231: inhibited

Storage
Dehydrated medium: 15-30°C
User prepared plates: 14 days at 2-8°C

References
Cain, D.B., Mc Cann, V.L. (1986) J. Clin. Microbiol. 23, 976
Curtis, G.D.W. et al. (1989) Lett. App. Microbiol. 8, 95
Donnelly, C.W., Baigent, G.J. (1986) App. Environ. Microbiol. 52, 689
FIL-IDF (1988) Provisional IDF recommended method Milk and Milk Products. Detection of *Listeria monocytogenes*.
ISO 10560: Milk and milk products-detection of *Listeria monocytogens.* 1993
Mc Clain, D., Lee, W.H. (1988) J. Ass. Off. Assol. Chem. 71, 660

LITMUS MILK

A differential medium for bacteriological test on dairy products

Code: KM3112

Typical formula (g/l)

Skim Milk	100.0
Litmus	0.75

pH 6.5 +/- 0.2

Directions

Mix 100g with a small quantity of cold distilled water, making a smooth paste and add more distilled water until a 10% mixture is obtained. Sterilise by steaming (100°C) on three successive days for 60, 45 and 80 minutes. Alternatively, autoclave at 121°C for 5 minutes.

Description

Litmus Milk can be used for the maintenance and cultivation of lactic acid bacteria. It is a differential medium used to determine several characteristics: lactose fermentation, caseolysis and casein coagulation. Litmus incorporated in the medium is both a pH and an oxidation-reduction indicator. Milk contains lactose and three main proteins: casein, lactalbumim and lactoglobulin. At pH 6.5 the medium is violet coloured; when inoculated with lactose-fermenting microorganisms, which produce lactic acid and occasionally butyric acid, it becomes pinkish red through the litmus reaction. Certain bacteria, which do not ferment lactose but attack peptones, with the production of ammoniun ions, produce a purple-blue colouration of the medium. Certain microorganisms remove the oxygen in the medium by means of a reductase, with reduction of the litmus to the leuco base coloured white. Coagulation of the medium can be produced by two distinct factors: 1) The lactic acid from lactose links with calcium caseinate to form caseinogen, which precipitates as an insoluble clot the rennin enzyme induces an alkaline coagulation of the medium, transforming the casein into procasein, in the presence of calcium ions. The peptonisation phenomenon is due to digestion of the casein, which manifests by clearing of the medium. Break-up of the coagulum indicates gas production by the microbial flora. For the detection of *C. perfringens* in water, inoculate freshly boiled tubes of litmus milk with various specimen quantities and heat to 80°C for 10-15 minutes to destroy non-spores. Incubate for 5 days at 37°C and observe daily for the typical coagulation reaction called “stormy clot".

The table below gives the culture characteristics of some microorganisms in Litmus Milk.

Microorganisms	**Culture characteristics**
Pseudomonas aeruginosa	peptonisation
Pseudomonas putida	alkaline reaction
Citrobacter freundii	acid reaction
Lactotacillus bulgaricus	acid reaction, clot
Lactotecillus acidophilus	acid reaction, clot
Clostridium perfringens	acid reaction and gas, clot
Listeria monocytogenes	reduction

Quality assurance (37°C-24hrs)
L.casei ATCC 7469: growth, acid reaction, and clot, pink ring
*P.aeruginosa ATCC 27853:*growth, peptonisation, clearing of medium

Storage
Dehydrated medium: 15-30°C

References
Mac Faddin, J.F. (1976) - Biochemical Tests for Identification of Medical Bacteria. Baltimore: The William and Wilkins Company.
Ministry of Health (1956) - Public Health Laboratory Service Water Committee. The Bacteriological Examination of Water Supplies. 3rd edition, HMSO, London.

LOWENSTEIN JENSEN MEDIUM BASE

A powdered medium for the detection of Mycobacteria

Code: KM3351

Typical formula	**(g/l)**
Magnesium Sulphate	0.24
Magnesium Citrate	0.60
Monopolassium Phosphate	2.50
L-Asparagine	3.60
Potato Flour	30.00
Malachite Green	0.40

Directions
Suspend 37.4g in 600 ml of distilled water, add 12ml of glycerol and heat to boiling. Autoclave at 121°C for 15 minutes. Cool to 50°C and add 1000ml homogenised whole eggs aseptically collected. Distribute into sterile tubes and heat to 75°C for 45 minutes in a slanting position until the medium solidifies due to coagulation of the egg.

Description
Lowenstein Jensen Medium Base is a medium used for the cultivation of Mycobacteria, especially *Mycobacterium tuberculosis*, from both clinical specimens and pure culture. The medium is prepared according to a Jensen modification of the original Lowenstein medium, and contains malachite green as a selective agent. The tubes are inoculated in duplicate, streaking the material to be examined onto the surface of the medium. Incubate at 37°C and examine after 10-14 days. *Mycobacterium tuberculosis* grows on the medium in large dry yellowish colonies. *Mycobacterium bovis* grows moderately in small colourless colonies.
For the cultivation of certain glycerol-sensitive strains of *Mycobacterium bovis*, glycerol must be omitted from the medium. In addition to Mycobacteria isolation, Lowenstein Jensen Medium can be used for the isolation of *Nocardia* from sputum, gastric fluid and other materials. Lowenstein Jensen Medium is recommended for testing Mycobacteria sensitivity to antibiotics. The drug sensitivity tests of Mycobacteria can be conducted according to one of the following methods:

1) Minimum inhibitory concentration
2) Resistance-ratio
3) Proportion

WHO experts emphasise that whatever the stage of development of the laboratory services of a country, no laboratory should embark on sensitivity testing until adequate staff, equipment, interest and intellectual skills are available to sustain a high standard of work. They also stress the necessity of centralising sensitivity tests in a few regional laboratories and of making one laboratory a national reference centre.

Storage
Dehydrated medium: 15-30°C
User prepared tubes: 3 weeks at 2-8°C in the dark

References
APHA (1963) - Diagnostic Procedure and Reagents. 4th edition.
Sommers, M.H. and J.K. Mc Clatcky (1983

Lauryl Sulphate Broth (LSB) X-GAL/MUG

A medium for the simultaneous detection of coliforms and *E. coli* with MPN chromogenic and fluorogenic method

Code: KM5411

Typical formula	**(g/l)**
X-GAL	0.08
IPTG	0.10
MUG	0.05
Sodium Chloride	5.00
Dipotassium Hydrogen Phosphate	2.75
Potassium Dihydrogen Phosphate	2.00
Tryptone	5.00
L-Tryptophan	2.00
Sorbitol	1.00
Sodium Lauryl Sulphate	0.10

pH 6.8 +/- 0.1

Directions
Suspend 18.1g in 1000ml of cold distilled water. Heat to complete dissolution and distribute into test tubes. Autoclave at 121°C for 15 minutes. For the examination of water prepare a multiple strength medium.

Description
LSB X-GAL/MUG is a modification of the original Lauryl Sulphate Broth formulation, studied for the simultaneous detection of coliform bacteria and *E. coli* in water and foodstuffs, with MPN method. The medium is partially inhibitory for Gram-positive bacteria by the presence of sodium lauryl sulphate; the differentiation of coliforms and among coliforms, of *E. coli*, is possible by the presence of the chromogenic substrate X-GAL (5-bromo-4-chloro-3 indolyl ß D-galactopyranoside), and the fluorogenic substrate MUG (4-methylumbellyferyl ß D-glucuronide). The first is cleaved by coliforms by means of ß-galactosidase with the release of a blue/blue-green pigment; the second is cleaved by *E. coli* with the beta-glucuronidase enzyme with the release of 4-methylumbelliferone, fluorescent under long-wave UV lamp. The beta galactosidase activity is amplified by the presence in the medium of IPTG (1-isopropyl-ß-d-tiogalactopyranoside). The tryptophan allows the confirmation of *E. coli* detection by means of the direct indole test, increasing the identification sensitivity.

Method
Use LSB X-GAL MUG referring to the various compendia for the examination of materials of sanitary importance. Incubate at 37°C for 24 - 48 hours. Expected results:

Total coliforms: blue/blue green growth
Escherichia coli: blue/blue-green growth, fluorescent under a Wood's lamp

To confirm the *E. coli*, add 1ml of Kovacs' Reagent to each tube: a red colour developing after 1-2 minutes is positive for indole test and confirms the presence of *E. coli*. **Notes:**

1. If the fluorescence emission is doubtful, add 0,5 ml of NaOH 0.5M to each tube and re-observe under a Wood's lamp
2. To examine samples strongly contaminated by Gram-positive bacteria, before sterilisation add 30mg of novobiocin (3 vials of Novobiocin Supplement to 1 litre of medium.

3 If a solid medium is preferred for the same diagnostic purposes, refer to C-EC Agar or Chromogenic Coliform Agar.

Quality assurance (37°C-24hrs)

Productivity control
E.coli ATCC 25922: blue growth, gas production, fluorescent under a Wood's lamp

Specificity control
C.freundii ATCC 43864: blue growth, gas production, and not fluorescent under a Wood's lamp

Selectivity control
E.faecalis ATCC 19433: partially inhibited, no gas production

Storage
Dehydrated medium: 15-30°C
User prepared tubes: 1 month at 2-8°C

References
Haynes, J.R., Covert, T.C., Rankin, C.C. (1993) Appl. Environ. Microb. 59, 2758
Manafi, M., Kneifel, W., Bascomb, S. (1991) Microbiol. Rev. 55, 335

LUXURIAN AGAR (EUGONIC)

A general purpose medium.

Code: KM6422

Typical formula	**(g/l)**
Tryptone	15.0
Soy Peptone	5.0
Sodium Chloride	4.0
Sodium Sulphite	0.2
L-Cystine	0.7
Glucose	5.5
Agar	15.0

LUXURIAN BROTH (EUGONIC)

A general purpose liquid medium.

Code: KM6432

Typical formula	**(g/l)**
Tryptone	15.0
Soy Peptone	5.0
Sodium Chloride	4.0
Sodium Sulphite	0.2
L-Cystine	0.7
Glucose	5.5

pH 7.0 +/- 0.2

Directions
Suspend 45.4g of Luxurian Agar or 30.4g of Luxurian Broth in 1000ml of cold distilled water. Heat to boiling, distribute and autoclave at 121°C for 15 minutes.

Description
Luxurian Agar and Broth are nutrient media, which support the luxuriant growth of most microorganisms, which are normally difficult to cultivate, including the pathogens *Haemophilus, Neisseria, Pasteurella, Brucella.* 5% (v/v) horse blood can be added to the media for the cultivation of *N. meningitidis, N.gonorrhoeae, Nocardia asteroides, Histoptasma capsulatum, Blastomyces dermatitidis.* Luxurian Agar can be used with the addition of 5% (v/v*)* horse blood and heated to 80°C for the preparation of chocolate agar. Luxurian Agar is also recommended for the detection of sporogenic microorganisms such as *Bacillus pumilus,* for the microbiological examination of meats and production processes, for the bacterial count of intestinal or oral flora specimens, and for the production of antigens and vaccines.

Quality assurance (24-48hrs/30°C)

Productivity control
S.aureus ATCC 25923: growth
E.coli ATCC 25922: growth

Storage
Dehydrated media: 15-30°C
User prepared plates and tubes: 1 month at 2-8°C

LYSINE IRON AGAR

A differential medium for the identification of *Enterobacteriaceae*

Code: KM5361

Typical formula	**(g/l)**
Peptone mixture	5.00
Yeast Extract	3.00
Glucose	1.00
L-Lysine	10.00
Fe-Ammonium Citrate	0.50
Sodium Thiosulphate	0.04
Bromocresol Purple	0.02
Agar	15.00

pH 6.7 +/- 0.2

Directions
Suspend 34.5g in 1000ml of cold distilled water; heat to boiling, distribute and autoclave at 121°C for 15 minutes. Cool in a slanting position so those deep butts are formed; inoculate by streaking and stabbing.

Description
Lysine Iron Agar is a differential medium for the detection of *Arizona* strains which rapidly ferment lactose and, more generally, to distinguish enteric bacteria on the basis of hydrogen sulphide production, and the decarboxylation and deamination of lysine. *Salmonella* and *Arizona* cause blackening of Bismuth Sulphite Agar, but some strains of *Arizona* which are frequently involved in cases of gastroenteritis of food origin rapidly ferment the lactose on T.S.I. causing strong acidification of the medium with consequent inhibition of hydrogen suiphide production. Lysine Iron Agar prepared without lactose permits detection of such strains of *Arizona.* The inclusion of lysine in the medium makes it possible to detect microorganisms that, through lysine decarboxylase activity *(Salmonella* and *Arizona),* cause alkalinisation of the medium. *Proteus* and *Providencia* deaminate lysine with a distinct red reaction in the slope of the tube and an alkaline reaction in the butt with purple colouration of the medium.

Method
Inoculate the strains by stabbing to the base of the butt and by streaking on the slope. The caps of the tubes must be replaced loosely so that aerobic conditions prevail on the slant. Incubate at 37°C for 18-24 hours. Purple colour: positive reaction: decarboxylation of lysine Yellow colour: negative reaction. Red colour: deamination of the lysine. Blackening of the medium: production of H_2S. Bubbles presences: gas production

The table below indicates the characteristic reactions of some *Enterobacteriaceae* on Lysine Iron Agar, after inoculation and incubation at 37°C for 24 hours.

Microorganism	**Slope**	**Butt**	**Gas**	**H_2S**
Escherichia	K	K o N	- o +	-
Salmonella typhi	K	K	-	-
Salmonella paratyphi	A	K	K+ o -	- o +
Salmonella arizonae	K	K o N	-	+ o –
Salmonella spp.	K	K	-	+
Proteus	*R*	*A*	-	- *(+)*
Providencia	*R*	*A*	-	-
Citrobacter	K	A	- o +	+

Shigella	K	A	-	-
Klebsiella	K	K	+ o -	-

K = purple alkaline reaction: decarboxylation of the lysine; N= no reaction; R = red colour: *deamination* of the lysine
A = yellow acid reaction: + black H_2S production

Quality assurance (37°C-24hrs)

Lysine decarboxylation control
S.enteritidis ATCC 13076: purple slope and butt, H_2S +

Lysine deamination control
P.mirabilis ATCC 25933: red slope, purple butt, H_2S +

Negative control
S.flexneri ATCC 12022

Storage
Dehydrated medium: 15-30°C
User prepared tubes: 1 month at 2-8°C

References
Edwards, P.R. & Ewing W.H. (1972) - Identification of Enterobacteriaceae, 3rd Ed. Minneapolis: Burgess Publishing Company.
Edwards, P.R. and Fife Marg. A. (1961) - App. Microbiol. 9, 478-480.

LACTALBUMIN HYDROLYSATE
A pancreatic Lactalbumin Hydrolysate

Code: PH1040

Lactalbumin Hydrolysate contains protein which has been reduced to its constituent amino acids and peptides. It is used as a microbiological nutrient for fermentations, production of biological products and in virus tissue cultures.

TYPICAL ANALYSIS
Total Nitrogen (TN) ..... 11-13 %
Amino Nitrogen (AN) ..... 4.5 - 6 %
Loss on drying .....< 6.5 %
Ash ..... < 7 %
pH (sol. 2 %) ..... 6.7 – 7.2
Solubility in water ..... 150 g/l

BACTERIOLOGICAL LACTOSE
Lactose Monohydrate

Code: CH1020

Bacteriological grade of lactose used in microbiological culture media as a fermentable carbohydrate.

TYPICAL ANALYSIS
Loss on drying ..... 5.3 %
pH 4.3
Ash..... 0.02 %
Heavy metals ..... < 5 ppm

LACTOSE BROTH

Used for the presumptive determination of coliforms in water and sewage

Code: KM1046

Typical formula	**(g/l)**
Beef Extract	3.0
Peptone Mix	5.0
Lactose	5.0

pH 6.9 +/- 0.2

Directions
Suspend 13g in 1000 ml of cold distilled water, heat to dissolve, distribute into fermentation tubes and autoclave at 121°C for 15 minutes. If necessary, use the medium at double or triple concentration.

Description
Lactose Broth is recommended for the presumptive determination of coliforms in water and sewage by the most probable number method; for the final confirmatory test of coliforms in dairy products after plating on Endo Agar or Levine EMS Blue Agar. It is also recommended by the FDA for the non-selective enrichment of *Salmonella*.
The medium contains lactose, meat extract and peptones in quantities that allow an optimal growth of non-fastidious micro-organisms such as the coliforms.

Method
For the presumptive determination of coliforms in water the following procedure is suggested. Inoculate a series of fermentation tubes with appropriate volumes of specimen in a way that does not alter the relationship between the final volume of the inoculated medium and the ingredients per litre. Any variation in the recommended work scheme given in the table below can alter the biological characteristics of the medium.

Inoculum ml	volume of medium ml/tube	Total volume medium + inoculum ml	Quantity of desired medium gr/litre
1	10 or more	1 or more	13
10	10	20	26
10	20	30	19.5
100	50	150	39
100	35	135	50.1
100	20	120	78

Incubate the fermentation tubes at 35°C and take the first reading by shaking the tube slightly after 24 hours. If gas production is not evident, incubate for a further 24 hours.
Gas formation within 48 hours is a positive indication of coliforms. The limit of 48 hours excludes the possibility of growing coliforms that ferment lactose slowly. These are of little sanitary interest and do not effect the validity of the method. To confirm the presumptive test, subculture the positive Lactose Broth tubes into tubes of Brilliant Green Bile Broth 2% The presence of coliforms in water is considered to be an indication of faecal pollution. Finding them in dairy products is an indication of inadequate control measures on productive cycles from a sanitary point of view and/or unsuitable forms of conservation.

Quality assurance (37°C-24hrs)

Productivity control
E.coli ATCC 25922: growth, gas production
C.freundii ATCC 43864: growth, gas production

Storage
Dehydrated medium: 15-30°C
User prepared tubes: 1 month at 2-8°C

References
APHA (1972) - Standard Methods for the Examination of Dairy Products, 13th Ed.
APHA (1985) - Standard Methods for the Examination of Water and Wastewater, 16th Ed.

LAURYL SULPHATE BROTH MUG

Used for the enumeration of *E. coli* and coliforms with fluorogenic MPN method, in milk and milk products.

Code: KM6412

Typical formula	**(g/l)**
Tryptose	20.00
Lactose	5.00
MUG	0.10
Sodium Chloride	5.00
Dipotassium Phosphate	2.75
Monopotassium Phosphate	2.75
L-Tryptophan	1.00
Sodium Laurylsulfate	0.10

pH 6.8 +/- 0.2

Directions
Suspend 36.7g in 1000ml of cold distilled water. Heat until complete dissolution and distribute into test tubes. Autoclave at 121°C for 15 minutes. Where necessary prepare a double strength medium.

Description
Lauryl Sulphate Broth MUG (LSB MUG) is a modification of Lauryl Sulphate Broth, for the detection of *E. coli* with fluorogenic MPN method, in milk and dairy products according to Standard IDF 140:1994. The presence of sodium lauryl sulphate makes the medium selective for; the differentiation of *E. coli* given by the presence of MUG (4-methylumbelliferil β D-glucuronide) which allows the detection of the β-glucuronidase enzyme. MUG is hydrolysed among enteric bacteria by *E. coli* and by few other strains of *Salmonella* and *Shigella*. Such hydrolyses occurs by release of 4-methylumbellyferone, fluorescent under a Wood's Lamp. The tryptophan contained in the medium allows you to perform the indole test directly on the tubes with the addition of Kovacs Reagent, for the *E. coli* confirmation.

Method
Prepare the test sample and the further dilutions according to Standard IDF 122B:1992 Take double and single strength medium tubes. Transfer 10ml of the test sample if liquid or 10ml of the primary dilution in case of solid sample, in tubes with double strength medium. Transfer 1ml of the test sample if liquid or 1ml of the primary dilution in case of solid sample, in tubes with single strength medium. For each of the further dilutions proceed in the same way. Carefully mix the inoculum with the medium. Incubate the tubes at 30°C for 24 +/ 2 hours. If neither gas formation nor opacity is observed, incubate for up to 48 +/ 2 hours. Perform the confirmatory test for *E coli*: add to each tube 0,5 ml of NaOH 0.1M and observe for fluorescence under a Woods Lamp; add to each tube 0.5 ml of Kovacs Reagent and observe for the formation of a purple ring. Identify those tubes, which develop fluorescence and are positive to indole test as *E. coli.* Identify those tubes, which show gas production as coliforms. For each dilution count the number of positive tubes and refer to MPN table to determine the MPN of microorganisms.

Quality assurance (37°C-24 hrs)

Productivity control
E.coli ATCC 25922: growth, gas production, fluorescent under Wood's lamp

Specificity control
C.freundii ATCC 43864: growth, gas production, and not fluorescent under a Wood's lamp

Selectivity control
E.faecalis ATCC 19433: partially inhibited, no gas production

Storage
Dehydrated medium: 15-30°C
User prepared tubes: 1 month at 2-8°C

Reference
IDF 170L (1994) Milk and Milk products. Enumeration of presumptive *E.coli.*

LAURYL SULPHATE BROTH MUG (+ Tryptophan)

For the enumeration of *E. coli* and coliforms with fluorogenic MPN method, in milk and milk products.

Code: KM8548

Typical formula	**(g/l)**
Tryptose	20.00
Lactose	5.00
MUG	0.10
Sodium Chloride	5.00
Dipotassium Phosphate	2.75
Monopotassium Phosphate	2.75
L-Tryptophan	1.00
Sodium Lauryl sulphate	0.10

pH 6.8 +/- 0.2

Directions
Suspend 36.7g in 1000ml of cold distilled water. Heat until complete dissolution and distribute into test tubes. Autoclave at 121°C for 15 minutes. Where necessary prepare a double strength medium.

Description
Lauryl Sulphate Broth MUG is a modification of Lauryl Sulphate Broth, for the detection of *E. coli* with fluorogenic MPN method, in milk and dairy products according to Standard IDF 140:1994. The presence of sodium lauryl sulphate makes the medium selective for; the differentiation of *E. coli* given by the presence of MUG (4-methylumbelliferil β D-glucuronide) which allows the detection of the β-glucuronidase enzyme. MUG is hydrolysed among enteric bacteria by *E. coli* and by few other strains of *Salmonella* and *Shigella*. Such hydrolyses occurs by release of 4-methylumbellyferone, fluorescent under a Wood's Lamp. The tryptophan allows the medium to perform the indole test directly on the tubes with the addition of Kovacs Reagent, for the *E. coli* confirmation.

Method
1. Prepare the test sample and the further dilutions according to Standard IDF 122B:1992
2. Take double and single strength medium tubes
3. Transfer 10ml of the test sample if liquid or 10ml of the primary dilution in case of solid sample, in tubes with double strength medium
4. Transfer 1ml of the test sample if liquid or 1ml of the primary dilution in case of solid sample, in tubes with single strength medium
5. For each of the further dilutions proceed in the same way
6. Carefully mix the inoculum with the medium
7. Incubate the tubes at 30°C for 24 +/ 2 hours
8. If neither gas formation nor opacity is observed, incubate for up to 48 +/ 2 hours
9. Perform the confirmatory test for *E coli*: add to each tube 0,5 ml of NaOH 0.1M and observe for fluorescence under a Woods Lamp;

add to each tube 0.5 ml of Kovacs Reagent and observe for the formation of a purple ring.

10. Identify those tubes which develop fluorescence and are positive to indole test as *E. coli*
11. Identify those tubes which show gas production as coliforms
12. For each dilution count the number of positive tubes and refer to MPN table to determine the MPN of microorganisms.

Quality assurance (37°C-24 hrs)

Productivity control
E.coli ATCC 25922: growth, gas production, fluorescent under Wood's lamp

Specificity control
C.freundii ATCC 43864: growth, gas production, and not fluorescent under a Wood's lamp

Selectivity control
E.faecalis ATCC 19433: partially inhibited, no gas production

Storage
Dehydrated medium: 15-30°C
User prepared tubes: 1 month at 2-8°C

Reference
IDF 170L (1994) Milk and Milk products. Enumeration of presumptive *E.coli.*

Lauryl Tryptose Broth

Code: KM1047

Description:

This medium is recommended for the detection of coliforms in dairy products, water and food samples. The excellent nutrient quality and phosphate buffer ensure the growth and gas production even in the case of slow lactose-fermenting coliforms or in the case of low concentration of inoculated bacteria. Sodium lauryl sulfate inhibits Gram-positive microorganisms.

Composition per litre:

Lactose……..5.00 g
Tryptose……..20.00 g
Sodium lauryl sulfate……..0.10 g
Dipotassium phosphate……..2.75 g
Monopotassium phosphate…….. 2.75 g
Sodium chloride……5.00 g

Final pH of the ready to use medium: 6.8 +/- 0.2

Medium preparation:

Add 35.6 grams of dehydrated culture medium to 1 liter of distilled water. Distribute into test tubes containing Durham tubes and autoclave for 15 min at 121ºC.

Quality specifications:

Dehydrated medium: homogeneous, light beige fine powder. Ready to use medium: yellow to gold and clear to trace hazy.

Microbiological response:

QC Results

Escherichia coli ATCC 25922 Growth, gas +
Proteus mirabilis ATCC 12453 Growth, gas -
Staphylococcus aureus ATCC 25923 No growth

Storage:

Dehydrated medium should be stored between 10 to 25ºC. Once opened, place the container in a dark, dry place. The dehydrated medium should not be used if there is any lump or if the colour has changed from the original.

LEGIONELLA SELECTIVE AGAR

A selective (BCYE/GVPC) and non-selective media for the isolation of *Legionella* spp.

Code: KM1048

Typical formula	**(g/l)**
Activated Charcoal	2.0
Yeast Extract	10.0
Agar	13.0

Supplemented with BCYE ά-Growth supplement (for 500ml of medium)**:**

ACES Buffer	5.0
Ferric pyrophosphate	125.0 mg
Cysteine	200.0 mg
α –ketoglutarate	0.5

pH 6.9 +/- 0.1

For selective Legionella Selective (GVPC) Agar add supplement (for 500ml of medium**):**

Glycine	1.5mg
Vancomycin	0.5mg
Polymixin B	39600 IU
Cycloheximide	40.0mg

pH 6.9 +/- 0.1

Directions
Weigh 12.5g in 450ml of distilled water. Heat to boiling with agitation and sterilise by autoclaving at 121ºC for 15 minutes. Cool to 50ºC and add the contents of one vial of BCYE α-Growth Supplement reconstituted with 50ml of sterile, warm distilled water (50ºC), then add the contents of GVPC selective Supplement reconstituted with 10ml of sterile distilled water. Mix well and distribute into sterile petri dishes.

Methods
Environmental samples:
Take 10ml of concentrated sample and centrifuge in sealed buckets at 2.500rpm for 20 minutes. Decant the supernatant and inoculate 0.1ml onto the selective agar plate. Leave approximately 1ml of fluid and with it re-suspend the sediment and inoculate 0.1ml onto the selective plate. To the re-suspended sediment add 9ml of HCl-KCl buffer pH 2.2 stir and leave at room temperature for 5 minutes. Inoculate 0.1ml onto the selective plate. HCl-KCl buffer pH 2.2: 3.9 ml of HCl 0.2M + 25ml of KCl 0.2 M – adjust to pH 2.2 with KOH 1M. Incubate the three plates at 37°C for 7 days and observe daily. Growth usually appears in 2-3 days.

Clinical samples:
Homogenise the lung tissue or bronchial aspirate in sterile distilled water. Examine microscopically for Legionella by FA method and for other bacteria with a Gram staining. Inoculate the specimens which are FA positive and without contaminants on a plate of Legionella Agar. Inoculate the specimens which are FA positive and with a contaminant flora on a plate of Legionella Selective Agar. Incubate the three plates at 37°C for 7 days and observe daily. Growth usually appears in 2-3 days.

The colony morphology of *Legionella* on the plating media after 48-72hrs of incubation is as follows:

L.pnemophila - diameter 1-2 mm (increase in size on further incubation), white, glistening, circular, smooth, raised with entire edge.
L.gormanii - diameter 1-2 mm, buff-white or cream, slight raised, mucoid.
Other *Legionella* - indistinguishable *from L.pneumophila*.

Select several colonies of each type and subculture onto a pair of plates, one of Blood Agar Sheep and the other of Legionella Agar. Regard as presumptive *Legionella* all colonies that grow on Legionella Agar and fail to grow on Blood Agar Sheep. Each *Legionella* presumptive colony should be confirmed serologically.

References

Edelstein, P.H. M.A.C.(1996) J.Clin.Microbiol, 34:185-187
Feeley J. et al (1978) J.Clin.Microbiol. 8, 320
Feeley J. et al (1979) J.Clin.Microbiol. 10, 437.

LETHEEN AGAR

For the determination of the antimicrobial activity of quaternary ammonium compounds.

Code: KM6419

Typical formula	**(g/l)**
Agar	20.0
Peptone mix	20.0
Beef Extract	3.0
Yeast Extract	2.0
Sodium Chloride	5.0
Lecithin	1.0
Tween 80	7.0
Glucose	1.0

pH: 7.2 + 0.2

Directions
Suspend 59g of Modified Letheen Agar in 1000ml of cold distilled water, heat to boiling, distribute and autoclave at 121°C for 15 minutes.

Description
Letheen Agar is prepared in accordance with the AOAC proposed formula, and is used to determine the phenol coefficient of quaternary ammonium disinfectants.

User quality assurance (24-48 hr at 30°C)

Productivity control
S.aureus ATCC 25923: growth
E.coli ATCC 25922: growth

Storage
Dehydrated medium: 15-30°C
User prepared tubes: 1 month at 2-8°C

Reference
AOAC (1995) - Official Methods of Analysis of AOAC International. 16th Ed.

LETHEEN BROTH

Used for the detection of phenol coefficient of quaternary ammonium disinfectants.

Code: KM6420

Typical Formula	**(g/l)**
Beef Extract	5.0
Peptone	10.0
Sodium Chloride	5.0
Lecithin	0.7

pH 7.0 + 0.1

Directions
Suspend 20.7g in 1000ml of cold distilled water, add 5g of polysorbate 80 (Tween 80), heat to boiling, distribute and autoclave at 121°C for 15 minutes.

Description
Letheen Broth is prepared in accordance with the AOAC proposed formula, and is used to determine the phenol coefficient of quaternary ammonium disinfectants. For use follow the AOAC recommended method.

Quality assurance (24-48 h /30°C)

Productivity control
S.aureus ATCC 25923: growth
E.coli ATCC 25922: growth

Storage
Dehydrated medium: 15-30°C
User prepared tubes: 1 month at 2-8°C

Reference
AOAC (1995) - Official Methods of Analysis of AOAC International. 16th Ed.

LISTERIA UVM BROTH BASE (+UVM1 & UVM2)

A selective broth base for the enrichment & detection of *Listeria monocytogenes* in meat and poultry.

Code: KM6972

Typical formula	**(g/l)**
Proteose Peptone	5.00
Tryptone	5.00
Meat Extract	5.00
Yeast Extract	5.00
Sodium Chloride	20.00
Sodium Phosphate Bibasic	12.00
Monopotassium Phosphate	1.35
Aesculin	1.00

pH 7.2 +/- 0.2.

Directions

Listeria UVM Broth Base:

Suspend 27.2g in 500ml of cold distilled water. Heat to boiling until complete dissolution. Autoclave at 121°C for 15 minutes.

Listeria UVM1 Enrichment Broth:

Dissolve the contents on one vial of Listeria UVM1 Antibiotic Supplement in 5ml of cold distilled water. Add to 500ml of autoclaved and cooled Listeria UVM Broth Base under aseptic conditions. Mix well and distribute in bottles or sterile tubes under aseptic conditions.

Listeria UVM 2 Enrichment Broth

Dissolve the contents on one vial of Listeria UVM2 Antibiotic Supplement in 5 ml of cold distilled water. Add to 500 ml of autoclaved and cooled Listeria UVM Broth Base under aseptic conditions. Mix well and distribute in bottles or sterile tubes under aseptic conditions.

Listeria Enrichment Broth UVM 1 and UVM2

Suspend 54.4g of Listeria Enrichment Broth UVM 1 or Listeria Enrichment Broth UVM 2 in 1000ml of cold distilled water. Heat to boiling until complete dissolution. Autoclave at 115°C for 15 minutes.

WARNING**:**

UVM1 and UVM2 Supplements and Listeria Enrichment Broth UVM 1 and UVM2 contain acriflavin, a possible mutagen. Do not inhale. In case of eye or skin contact wash affected area thoroughly with soap and water.

Description

UVM Liquid media are prepared according to the formulations described by Donnelly and Baigent and by MacCalin and Lee. UVM1 and UVM2 media are used for the “two steps” enrichment of *Listeria* spp. in meat products. The complete UVM1 medium contains a concentration of 20mg/l of nalidixic acid and 12 mg/ltr of acriflavin; the complete UVM2 medium contains a double concentration of acriflavin (25mg/ltr) respect to UVM1 medium.

Method

1. Add 225ml of UVM1 Enrichment Broth to 25g or 25ml of sample. Homogenise for 2 minutes. Incubate at 30°C for 24 hours.
2. After 4 hours incubation spread 0.2ml onto Listeria Selective Agar Plates.
3. After 24 hours add 1ml to 4.5ml of sterile KOH solution (2.5g KOH and 20g NaCl in 1000ml of distilled water: pH over 12). Vortex mix one minute and within one minute subculture onto Listeria Selective Agar Plates. After 24 hours transfer 0.1ml of Listeria UVM1 culture to 10ml of Listeria UVM Broth Base supplemented with UVM2 Supplement.
4. Incubate the Secondary Enrichment Medium at 30°C for 24 hours. After 24 hours incubation proceed as follows: Spread 0.2ml of Secondary Enrichment Medium onto Listeria Selective Plates. Add 1ml to 4.5ml of sterile KOH solution and proceed as point 3.

Incubate the Selective Agar plates at 37°C for 24-48 hours, examine for typical colonies and carry on with identification tests by means of standard biochemical method. **Note**: Techniques for the detection of *Listeria* in foods vary, depending on the material under examination and local laws. Refer to various compendia or to national regulations for the complete procedures.

Quality assurance (37°C-24 hrs)

Productivity control
L.monocytogenes ATCC 19117: growth

Selectivity control
S.aureus ATCC 25923: inhibited

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

References
Cain, D.B., Mc Cann, V.L. (1986) J. Clin. Microbiol. 23, 976
Connelly, C.W., Baigent, G.J. (1986) App. Environ. Microbiol. 52, 689
Curtis, G.D.W. et al. (1989) Lett. App. Microbiol. 8, 95
Haley, L.D., Trandel, J.B., Coyle, M.B. (1980) Pratical methods for culture and identification of fungi in the clinical microbiological laboratory. Cumitech n. 11, ASM, Washington, D.C.
McClain, D., Lee, W.H. (1988) J. Ass. Off. Anal. Chem. 71, 660
Martindale The Extra Pharmacopoeia (1982) Twenty-eighth Edition. The Pharmaceutical Press, London.

Listeria Agar (C.O.A.L.A.)

Powdered medium base and selective supplement for the resuscitation, direct enumeration and identification of *Listeria monocytogenes* in foodstuffs

Media code: KM6052
Supplement code: KMS3501C

TYPICAL FORMULA C.O.A.L.A. (g/ltr)

Peptones	54.50
Phosphate buffer	5.00
Growth factors	3.30
Sodium chloride	3.50
Lithium chloride	10.00
Selective compounds	0.06
Chromogenic compounds	0.05
Agar	15.00

C.O.A.L.A. Supplement (g/vial)
Selective compounds 0.1

Final pH: 7.2 ± 0.2

Directions
Suspend 44g in 500 ml of cold distilled water; heat to boiling and sterilise in the autoclave at 121ºC for 15 minutes. Cool to 48-50 °C and add the contents of one vial of C.O.A.L.A. Supplement reconstituted with 5 ml of sterile distilled water. Mix well and pour into inoculated Petri dishes. Aspect of the medium: homogeneously turbid.

Description
C.O.A.L.A. Listeria Agar has been developed modifying the ALOA medium. C.O.A.L.A. is suitable for the rapid enumeration of *Listeria monocytogenes* in raw foods (especially grinded raw meat, meat products, cooked, fermented, salted intermediate products used for the production of meat products, frozen foods (vegetables, meat and fish products, confectionery products), foods to be cooked before they are consumed (cold roast) .
The enumeration of *L.monocytogenes,* without the enrichment step, has a sensitivity of 10 cells/g by inoculating 1 ml of sample suspension into a 90 mm Petri dish or a sensitivity of 1 cell/g by inoculating 5+5 ml of sample suspension into two 150 mm Petri dishes with 30 ml of culture medium each.

The advantages of using C.O.A.L.A Medium in respect to other *Listeria* plating media are the follows:

- Resuscitation of the stressed cells because of the use of a high peptones concentration and especially selected growth factors.
- Differentiation of *L.monocytogenes* from other *Listeria* spp. by means of a chromogenic mixture and the use of a specific, purified substrate for a lipase enzyme that is present in *L. monocytogenes* colonies only. The high specificity of this enzymatic reaction allows to identify the colonies cultivated with red colour and with an opaque halo after 24 hours of incubation as *L.monocytogenes*.
- Selective activity due to lithium chloride and to a mixture of antibiotics, including ceftazidime.
- C.O.A.L.A. Medium gives no false negative results and very few false positive results. C.O.A.L.A. Medium is a useful tool in the laboratories where rapid and accurate results

are needed and not bound to use of standard methods (ISO, FIL or other methods given by regulatory bodies).

Technique

Liquid samples

Take two sterile Petri dishes and transfer, by means of a sterile pipette, 1ml of the test sample in each dish.
Pour about 15ml of C.O.A.L.A Medium cooled to 46-49°C into each Petri dishes.
Carefully mix the inoculum with the medium by rotating the plates and allow the plates to solidify on a cool horizontal surface.

Solid samples

Prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerned. ISO 6887 recommends the use of peptone salt - see Maximum Recovery Diluent, as general diluent for foods and animal feeding stuffs. Take two sterile Petri dishes, 150 mm in diameter and transfer, by means of a sterile pipette, 5 ml of the initial suspension to each dish . In this way 1 g of sample is inoculated into 2 dishes. Pour about 30 ml of C.O.A.L.A Medium cooled to 46-49°C into each Petri dishes. Carefully mix the inoculum with the medium by rotating the plates and allow the plates to solidify on a cool horizontal surface. Invert the prepared plates and place them in the incubator at 37°C. Examine the plates after 24 +/- 2 hours of incubation. Count as *L. monocytogenes* the magenta-red colonies surrounded by an opaque halo (typical colonies). Consider as *Listeria* sp. non-*monocytogenes* the magenta-red colonies without the opaque halo. If no typical colonies are present or no growth occurs after 24 h of incubation, re-incubate the plates for further 18-24 hours. If no typical colonies develop, the sample can be considered *L.monocytogenes* free at the sensitivity level chosen (1 or 10 cells/g). If typical colonies grow in the second period of incubation, confirm these colonies with the rapid test. After 2 hours of incubation the inoculated paper discs will turn to yellow if the colonies are *Listeria* non-*monocytogenes*; the discs remain colourless or light pink if the colonies are *L.monocytogenes*. Discharge the dishes with the colonies positive to the disc test (yellow colour). Consider the colonies negative to the disc test as *L.monocytogenes*. The confirmation test of the typical colonies grown after 48 hours is needed because some strains of *Listeria ivanovii* may produce a rather vague opaque halo, especially after 48 hr incubation. Count as *L.monocytogenes* the colonies confirmed with the test “Monocytogenes ID Disc” and express the results as CFU/g.

User quality assurance (37°C –24 h)

Productivity control

L.monocytogenes ATCC 19111: growth, magenta-red colonies surrounded by an opaque halo

Specificity control

L.innocua ATCC 33090: growth, magenta-red colonies without opaque halo

Selectivity control

E.coli ATCC 25922 : inhibited
E.faecalis ATCC 19433: inhibited
C.albicans ATCC 10231: inhibited

Storage

Dehydrated medium: 2-8°C

LISTERIA BUFFERED ENRICHMENT BROTH

A selective buffered enrichment broth for detection of *Listeria* spp.

Code: KM6139

Typical formula	**(g/l)**
Tryptone Soy Broth	30.00
Yeast Extract	6.00
Monopotassium phosphate	1.35
Disodium phosphate	9.60
Cycloheximide	0.05
Nalidixic Acid	0.04
Acriflavin HCl	0.015

pH 7.3 +/- 0.2

Directions
Suspend 47g in 1000ml of cold distilled water. Heat to dissolve, distribute and sterilise by autoclaving at 115°C for 15 minutes.

WARNING: The medium contains acriflavine, a possible mutagen and cycloheximide, a skin irritant. Do not inhale. In case of eye or skin contact wash affected area thoroughly with soap and water.

Description
Buffered Listeria Enrichment Broth is based on the typical formulation described by J.Lovett for the enrichment *of L. monocytogenes* in foodstuffs modified by increasing its buffering strength. Preliminary collaborative and other studies showed that the degree of buffering is not crucial in a 2-day enrichment when conventional isolation and identification methods are used. Nevertheless, rapid DNA probe methods require more stringent control of pH to counteract effects of competitors. It contains cycloheximide, acriflavine and nalidixic acid as antibacterial and antifungal drugs. Because all these agents are thermostable they are included in the powdered medium and can be sterilised by autoclaving.

Method
Add 25g of sample to 225ml of Buffered Listeria Enrichment. Blend until the test portion is thoroughly dispersed. Incubate the inoculated enrichment medium for 48hrs at 30°C. Streak a loopful of the enrichment culture onto a surface of the Listeria Isolation Agar Palcam Agar. Incubate at 37°C for 24 hours. Examine for the presence of typical colonies. Carry on with suitable identification tests. **Note:** Techniques for the detection of *Listeria* in foods vary, depending on material under examination and local laws. Refer to various compendia or to national regulations for the complete procedures.

Quality assurance (37°C-24hrs)

Productivity control
L.monocytogenes ATCC 19117: growth

Selectivity control
S.aureus ATCC 25923: inhibited

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

References
FDA (1995) Bacteriological Analytical Manual, 8th ed. Revision A, 1998. Published by AOAC International.
Lovett, J., Francis D.W. and Hunt J.M. (1987) J. Food Prot. 50,188-192

LISTERIA ENRICHMENT BROTH

A selective broth for the enrichment of *Listeria* spp.

Code: KM1050

Typical formula	**(g/l)**
Tryptone	15.00
Soy Peptone	3.00
Yeast Extract	6.00
Cycloheximide	0.05
Nalidixic Acid	0.04
Acriflavin HCl	0.015
Sodium Chloride	5.00
Dipotassium Phosphate	2.50
Glucose	2.50

pH 7.3 +/- 0.2

Directions
Suspend 36g in 1000 ml of cold distilled water. Heat to dissolve, distribute and sterilise by autoclaving at 115°C for 15 minutes.

WARNING: This medium contains acriflavine, a possible mutagen and cycloheximide, a skin irritant. Do not inhale. In case of eye or skin contact wash affected area thoroughly with soap and water. Safety Data Sheet available with this medium.

Description
Listeria Enrichment Broth is based on the typical formulation described by J.Lovett for the enrichment of *L. monocytogenes* in foodstuffs. It contains cycloheximide, acriflavine and nalidixic acid as antibacterial and antifungal drugs. Because all these agents are thermostable they are included in the powdered medium and can be sterilised by autoclaving.

Method
Add 25g of sample to 225 ml of Listeria Enrichment Broth. Blend until the test portion is thoroughly dispersed. Incubate the inoculated enrichment medium for 48hrs at 30°C. Streak a loopful of the enrichment culture onto a surface of the Listeria Isolation Agar or Palcam Agar. Incubate at 37°C for 24 hours. Examine for the presence of typical colonies. Carry on with suitable identification tests.
Note: Techniques for the detection of *Listeria* in foods vary, depending on the material under examination and local laws. Refer to various compendia or to national regulations for the complete procedures.

Quality assurance (37°C-24hrs)

Productivity control
L.monocytogenes ATCC 19117: growth

Selectivity control
S.aureus ATCC 25923: inhibited

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

References
Haley, L.D., Trandel, J.B., Coyle, M.B. (1980) Pratical methods for culture and identification of fungi in the clinical microbiological laboratory. Cumitech n. 11, ASM, Washington, D.C.
Lovett, J. (1986) Protocollo FDA 08/08/1986
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