ENDO BROTH

A selective medium for the enumeration of coliforms in water and milk by the membrane filter method.

Code: KM6612

Typical formula	**(g/l)**
Tryptone	5.00
Bacto Peptone	5.00
Soy Peptone	10.00
Yeast Extract	1.50
Lactose	12.50
Sodium Chloride	5.00
Dipotassium Phosphate	4.375
Monopotassium Phosphate	1.375
Sodium Sulphite	2.100
Sodium Desoxycholate	0.100
Sodium Lauryl Sulphate	0.050
Basic Fuchsin	1.050

pH 7.2 +/- 0.2

Directions
Suspend 48g in 1000ml of cold distilled water containing 20ml of ethanol. Mix and heat to boiling, but do not over boil or sterilise in the autoclave. The medium should be used the day it is prepared: if necessary store in the dark at 4 °C for not more than 96 hours. Basic fuchsin is a potential carcinogen and care must be taken to avoid inhalation of the powdered medium and contamination of the skin.

Description
Endo Broth is a selective medium for the enumeration of coliforms in water and milk by the membrane filter method. For the technique, see APHA indications. Coliforms grow turning the medium purple, with or without a metallic sheen. The volume of specimen for filtration should be selected according to the number of expected colonies. The ideal quantity should supply a microbial growth of 50 to 200 colonies. With the exception of drinking water and swimming pool water, which must be filtered in duplicate to a volume of 100 or 500 ml, all other water specimens are filtered at three different volumetric levels, diluted and undiluted.
Preliminary enrichment of the specimens is unnecessary in the case of non-drinking water and wastewater. In the case of drinking water, preliminary enrichment of the specimen gives excellent results, although it is not indispensable for the routine examination of specimens. For correct specimen enrichment procedure, see quoted literature.

Quality assurance (37°C-24 h)

Productivity control
E.coli ATCC 25922: growth, metallic colonies
E.aerogenes ATCC 25922: growth, red colonies
S.enteritidis ATCC 13076: growth, colourless colonies

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

Reference
APHA (1985). Standard Methods for the Examination of Water and Wastewater. 16th edition

E. coli Direct AGAR MUG

A selective medium for the detection of *E.coli* in water and food with MF procedure

Code: KM3272

Typical formula	**(g/l)**
Tryptone	20.0
Yeast Extract	5.0
Bile Salts No. 3	1.5
Disodiurn Phosphate	5.0
Monopotassium Phosphate	1.5
Sodium Chloride	5.0
MUG	0.1
Agar	12.0

pH 7.2 +/- 0.2

Directions
Suspend 50g in 1000ml of cold distilled water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes.

Description
E. coli Direct AGAR MUG is a selective and differential medium for isolation, enumeration and identification of *E.coli.* The selective action is obtained with the presence of bile salts, which inhibit the growth of Gram-positive bacteria. *E. coli* Direct AGAR MUG does not contain lactose and, therefore, the substrate is not acidified during *E.coli* growth. The obtained fluorescence in this way, having a maximum intensity through an alkaline pH, is more evident than on other media with MUG. *E. coli* Direct AGAR MUG is prepared with tryptophan-rich peptones in such a way that it is possible the direct determination of indole on the colonies. This medium is recommended for isolation, enumeration and direct identification of *E.coli* using the membrane filtration technique.

Method
Place 10ml of the sample in a filtration device with a 0.45 micron membrane. Lay the membrane on a Tryptone Soy Agar plate and pre-incubate for 2-4 hours at 37°C or overnight at 20°C. Transfer the membrane onto the *E. coli* Direct AGAR MUG plate and incubate overnight at 44°C. Observe under Wood's lamp (366 nm) for the presence of fluorescent colonies. Lay a drop of Kovacs' Reagent on the colonies. MUG positive and indole positive colonies are enumerated and identified as *E.coli.*

User quality assurance (37°C-24 hrs)

Productivity control
E.coli ATCC 25922: growth, fluorescent colonies under Wood's lamp
C.freundii ATCC 43864: growth, not fluorescent colonies under Wood's lamp

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

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

EC BROTH

A liquid medium used for the detection of faecal coliform organisms in waters and foodstuff

Code: KM3252

Typical formula	**(g/l)**
Tryptone	20.0
Lactose	5.0
Dipotassium Phosphate	4.0
Monopotassium Phosphate	1.5
Sodium Chloride	5.0
Bile Salts No. 3	1.5

pH 6.9 +/- 0.2

Directions
Suspend 37g in 1000ml of cold distilled water, heat to dissolve, distribute into fermentation tubes and sterilise at 121°C for 15 minutes. Inoculate not more than 1ml of sample in 10ml of medium, or use multiple strength medium.

Description
EC Broth is prepared according to the formulation proposed by Hajna and Perry and reported by APHA, ICMSF and ISO 7251. EC Broth is lactose buffered broth, with bile salts to obtain the inhibition of Gram-positive cocci and spore-forming organisms, which are often responsible for false positive results in Lactose Broth or Lauryl Sulphate Broth. The medium is recommended by APHA for the detection of faecal coliform organisms in waters and foodstuff, together with Faecal Coliform Broth, which can be mainly applied to treated waters and sea water and by ISO 7251, for the enumeration of *E.coli* with MPN technique.

Method
ISO method for *E.coli*: Prepare the test sample and the decimal dilution in accordance with the specific Laboratory method using Maximum Recovery Diluent or another suitable diluent. Take three tubes of double-strength Lauryl Peptone Broth and three tubes of single-strength Lauryl Peptone Broth and by means of a sterile pipette transfer to each tube 10ml or 1ml respectively of the test sample, if liquid or 10ml or 1ml of the initial suspension in the case of other products. Repeat the inoculation of the single strength and the double strength liquid medium for each of the further decimal dilutions, using a fresh pipette for each dilution. Incubate the tubes at 35- 37°C for 24 +/- 2 hours. From each of the incubated tubes showing gas production inoculate with a loop a tube of EC Broth and incubate at 45°C for 24 +/- 2 hours. From each of the EC Broth tubes showing gas production inoculate with a loop a tube of Peptone-Tryptone Water and incubate at 45°C for 48 hours. Carry on the indole test by adding 0.5 ml of Kovacs' Reagent Express the results as the Most Probable Number of *E.coli* on the basis of gas production in the EC Broth and the positive result to indole test, in Peptone Water. APHA method for faecal coliforms: Transfer by means of 3mm loop, from all the tubes of Lauryl Peptone Broth showing gas formation within 24 hours and from all the tubes showing growth within 48 hours, to EC Broth tubes. Within 30 minutes from the inoculum, place the tubes in a water-bath and incubate at 44°C for 24 hours. Consider the tubes showing gas production as positive. Calculate the density of the faecal coliform organisms by using MPN tables.

Quality assurance (44°C - 24 hrs)

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

Selectivity control
P.aeruginosa ATCC 27853: inhibited

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

References
APHA (1985) Standard Methods for the Examination of Water and Wastewater, 16th Ed., pp. 878-882.
APHA (1985) Compendium of Methods for the Microbiological Examination of Foods, 2nd Ed.
ISO 7251 Microbiology - General Guidance for enumeration of presumptive *E. coli* - Most Probable Number Technique. 2nd Ed. 1993 - 12 - 15
Perry and Hajna (1943) Am. J. Pub. Hlth., 33, 550.

E.E. BROTH

Code: KM1029

A liquid culture medium for the enrichment of enterobacteria from food samples.

Typical formula	**(g/l)**
Gelatin peptone	10.00
Dextrose	5.00
Ox bile	20.00
Di-sodium phosphate	6.45
Mono potassium phosphate	2.00
Brilliant green	0.015

pH 7.2 ± 0.2

Directions
Suspend 43.5 g of powder in 1 litre distilled water and heat until dissolution. Sterilise at 121°C for 5 minutes or boil it for 30 minutes. Cool quickly. OVERHEATING THIS MEDIUM WILL ADVERSELY AFFECT ITS PERFORMANCE.

Description
This medium is for enterobacteria enrichment and it is a Mossel's modification (1963) to the classic Brilliant Green Bile 2% Broth. Substitution of lactose by glucose makes it more suitable for enteric bacteria detection, even gas producer or non-gas-producer, in foods and different samples. The recommended technique is as follows: sample to study is added to sterile broth at 10% proportion. After a strong homogenisation the mixture is incubated for 18-20 hours period at 35-37°C. Following, subcultures are performed on a solid media appropriate to the selective enterobacteria isolation. For this step, it is especially suggested Violet Red Bile Agar though there are also the MacConkey, Desoxycholate or Brilliant green based media. For suspicious colonies on this media identification will be performed following the most common methodology.

References
MOSSEL, VISSER and CORNELISSEN (1973) J. Appl.Bact. 26:444

ENDO AGAR

A medium for confirmatory and final tests of coliforms in water, dairy products and foodstuffs

Code: KM1030

Typical formula	**(g/l)**
Tryptone	10.0
Lactose	10.0
Dipotassium Phosphate	3.5
Sodium Sulphite	2.5
Basic Fuchsin	0.4
Agar	15.0

pH 7.5 +/- 0.2

Directions
Suspend 41g in 1000ml of cold distilled water, heat to boiling and autoclave at 121°C for 15 minutes. Swirling the flask containing the medium before filling the petri dishes can eliminate the flocculent precipitates characteristic of Endo Agar. Basic fuchsin is a potential carcinogen and care should be taken to avoid inhalation of the powdered medium and contamination of the skin. Plates should be stored in the dark to preserve their pale pink colour.

Description
Endo Agar is a selective and differential medium used in the past for the isolation of *Salmonella typhi*, and was recommended by ICMSF and APHA for confirmatory and final tests (completed test) of coliforms in water, dairy products and foodstuffs. Coliforms grow on Endo Agar with red-violet colonies, with or without a metallic sheen. Non lactose-fermenting bacteria grow with colonies of the same colour as the medium, and have a tendency to fade to a faint pink colour. Gram-positive bacteria are inhibited by the presence of the sodium sulphite and basic fuchsin in tests carried out on water and dairy products. APHA recommends the subculture of the positive Brilliant Green Bile 2% Broth tubes, used in the confirmatory coliform tests, onto Endo Agar (or, Levine EMB Blue Agar) plates, streaking the surface of the medium with a loop in such a way that a distance of at least 0.5cm is kept between one colony and the next. After incubation at 35°C for 24 hours, the Brilliant Green Bile 2% Broth cultures, which have been transferred onto Endo Agar, are considered to be coliform positive if they have given rise to red colonies with or without a metallic sheen. In microbiological assays on foodstuffs, ICMSF recommends Endo Agar for confirmatory coliform tests together with Levine EMB Blue Agar and Brilliant Green Bile 2% Broth. They also suggest the following procedure: transfer a loopful of microbial growth from positive Lauryl Peptone Broth tubes, used in the presumptive test, onto Endo Agar plates. Incubate at 35°C for 48 hours. The Lauryl Peptone Broth cultures, which give rise to colonies with typical coliform characteristics on Endo Agar, are considered to be positive.

Quality assurance (37°C-24 hrs)

Productivity control
E.coli ATCC 25922: growth, red colonies with greenish metallic sheen
E.aerogenes ATCC 25922: growth, red colonies
S.enteritidis ATCC 13076: growth, colourless colonies

Storage
Dehydrated medium: 15-30°C
User prepared plates: 3 days at 2-8°C

References
APHA (1972), Standard Methods for the Examination of Dairy Products, 13th edition
APHA (1980), Standard Methods for the Examination of Water and Wastewater, 15th edition
ICMSF (1978). Microorganisms in Food: their Significance and Methods of Enumeration, 2nd edition.

Eosin Methylene Blue Agar (Levine)

Recommended for the isolation and differentiation 0f enteric bacilli.

Code: KM1031

Typical formula	**(g/l)**
Peptone	10.0
Lactose	10.0
Dipotassium phosphate	0.7
Monopotassium phosphate	1.3
Eosin Y	0.4
Methylene Blue	0.065
Agar	15.0

pH: 6.8 ± 0.2

Directions
Weigh 37.5 grams of powder, disperse in 1 litre of deionised water. Allow to stand for 10 minutes, swirl to mix then sterilise by autoclaving at 121ºC for 15 minutes. Cool to 50ºC and agitate gently to ensure uniform distribution of the flocculant precipitate, which is a feature of this medium before pouring into petri dishes. STORE IN THE DARK.

Description
This medium was introduced in 1916 by Holt-Harris and Teague to differentiate Escherichia spp. and Aerobacter spp. It was modified by Levine in 1918 who removed sucrose from the formula and increased the lactose content. The distinctive metallic sheen produced by E. coli on this medium is due to acid production resulting in an amide bonding between the eosin and methylene blue, other coliforms do not produce enough acid to cause this reaction. Eosin inhibits most Gram positive organisms. The prepared medium is sensitive to light.

Appearance: Blue/purple with a light precipitate.

Quality Assurance organisms: *E. coli,* Klebsiella spp

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

Inoculation: Surface, streaking for single colonies.

Incubation: 37ºC aerobically for 24 hours.

References
American Public Health Association, American Water Works Association and Water Pollution Control Federation, 1975. Standard Methods for the Examination of Water and.
Levine, M. 1918. Differentiation of E. coli and B. aerogenes on a simplified Eosin-Methylene Blue agar. J. Infect. Dis., 23: 43-47.

EVA (ETHYL VIOLET AZIDE**) BROTH**

A selective medium for the detection of enterococci

Code: KM9888

Typical formula	**(g/l)**
Tryptose	20.00
Sodium Chloride	5.00
Glucose	5.00
Dipotassium Phosphate	2.70
Monopotassium Phosphate	2.70
Sodium Azide	0.40
Ethyl Violet	0.83 mg

pH 7.0 +/- 0.2

Directions
Suspend 35.8g in 1000ml of cold distilled water, heat to dissolve completely, distribute and autoclave at 121°C for 15 minutes.

Description
Ethyl Violet Azide Broth is a selective medium for the detection of enterococci in water, as a faecal pollution indicators. It is used for the confirmatory test of enterococci, sub-culturing from the tubes of Azide Dextrose Broth. The medium is a modification of the original formula of Litsky et al. Together with the ethyl violet, the sodium azide makes the medium selective for enterococci by inhibiting all other Gram-positive and Gram-negative bacteria.

Method
Incubate the inoculated tubes of Azide Dextrose Broth at 37°C for 24-48 hours. Transfer three loopfuls of microbial growth from the positive tubes of Azide Dextrose Broth, into tubes containing 10 ml of Ethyl Violet Azide Broth, and incubate for 24 hours at 37°C. The presence of enterococci is shown by a clouding of the broth and the formation of a purple ring on the bottom of the tubes.

Quality assurance (37°C-48 hr)

Productivity control
E.faecalis ATCC 29212: growth

Selectivity control
E.coli ATCC 25922: inhibited
S.pyogenes ATCC 19615: inhibited

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

References
APHA (1976), Standard Methods for the Examination of Water and Wastewater, 14th edition
Litsky, W., Mallmann, W.L. & Fifield, C.V. (1953) Amer. J. Pub. Hlth. 43, 873.

EXTRACTS

Beef Extract Powder
EX1019

This complements the nutritive properties of peptones in culture media and is often used as an added enrichment. Beef extract can be used as a direct replacement for meat peptones and, as it contains no carbohydrates, can be used as a component of media for fermentation studies.

Typical Analysis

Appearance: light brown powder
Solubility in water at 5% total
Clarity clear, light brown colour
pH of 2% solution 7.0 ± 0.2
Total Nitrogen 12.0% ± 0.5
Amino Nitrogen 1.6% ± 0.5

Malt Extract Powder
EX1023

A water-soluble extract of malted barley suitable for use in the cultivation of yeasts and moulds. Malt extract has a very high carbohydrate content and consequently is very sensitive to over heating.

Typical Analysis

Appearance: yellow/brown powder
Solubility in water at 5% total
Clarity clear, light brown colour
pH of 2% solution 5.2 ± 0.2
Maltose 55%
Other Carbohydrates 40%
Protein 5%

Meat Extract Powder
Code: EX1030

Similar to Beef Extract this complements the nutritive properties of peptones in culture media and is often used as an added enrichment. Meat extract can be used to replace meat peptones and can be used for fermentation.

Typical Analysis

Appearance: brown/beige powder
Solubility in water at 5% total
Clarity clear, light brown colour
pH of 2% solution 7.0 ± 0.5
Total Nitrogen 15.0% ± 0.5
Amino Nitrogen 2.5% ± 0.5

Yeast Extract Powder
Code: EX501

Prepared by the autolysis of Saccharomyces cerevisae under thermostatically controlled conditions to protect the B vitamins and other heat labile constituents. This extract provides a mixture of amino acids, peptides, vitamins and carbohydrates making it suitable for many applications.

Typical Analysis

Appearance yellow powder
Solubility in water at 5%total
Clarity clear Beige/yellow

LIVER EXTRACT (Dehydrated liver extract)
Code: EX1031

Liver extract is a dehydrated and standardised extract from beef liver used for the preparation of infusion media. It is useful for the culture of *Trichomonas vaginalis* and other fastidious protozoa, for those culture media designed to target pathogenic fungi & anaerobic bacteria.

Typical Analysis

Total Nitrogen (TN) ..... 11.8 %
Amino Nitrogen (AN) ..... 5.6 %
Loss on drying ..... 4.1 %
Ash..... 15.5 %
pH (5 % solution) 6.8