MACCONKEY BROTH (PURPLE)

For the detection and enumeration of faecal coliforms (37ºC) and *E. coli* (44ºC) in water, dairy products and foodstuffs

Code: KM1057

Typical formula	**(g/l)**
Peptone	20.00
Lactose	10.00
Bile Salts	5.00
Bromocresol Purple	0.01

pH 7.3 +/- 0.2

Directions
Suspend 35g in 1000ml of cold distilled water, heat to dissolve, distribute into fermentation tubes and autoclave at 121°C for 15 minutes. With inocula greater than 1ml for 10ml of medium, use a multiple strength medium.

Description
MacConkey Broth Purple is used for the enumeration of coliforms in water, dairy products and foodstuffs. The litmus originally used as an indicator was substituted with neutral red. Because of the inhibitory effects of neutral red on *Escherichia coli* growth, the use of bromocresol purple was proposed. This indicator is less selective than neutral red, and more sensitive in recording pH variations in the medium.

Method
Liquid specimens are inoculated into the medium as they are, whereas solid ones should be homogenised. The inoculation must be effected at 10% *(v/v)* in Durham tubes. If the inoculum is greater than 1ml it is necessary to use the medium at double strength, inoculating equal volumes of specimen and medium Coliform presence is shown by the appearance of a yellow colour and the production of gas, which collects at the top of the Durham tube after 24-48 hours of incubation at 35°C.

Quality assurance (37°C - 24hrs)

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

Selectivity control
S.aureus ATCC 25923: inhibited

Storage
Dehydrated medium: 15-30°C
Prepared tubes: 15 days at 2-8°C

References
APHA (1985) - Compendium of Methods for the Microbiological Examination of Foods, 2nd Ed.
APHA (1985) - Standard Methods for the Examination of Water and Wastewater. 16th edition.

MacCONKEY SORBITOL AGAR

A selective and differential medium, with sorbitol, for the detection of *E.coli* O157

Code: KM1058

Typical formula	**(g/l)**
Peptone mix	25.00
D-Sorbitol	10.00
Bile Salts No. 3	1.50
Sodium Chloride	5.00
Neutral Red	0.030
Crystal Violet	0.001
Agar	14.50

pH 7.1 +/- 0.2

MacCONKEY SORBITOL MUG AGAR

A selective and differential medium, with sorbitol and MUG, for the detection of *E.coli* O157

Code: KM1058M

Typical formula	**(g/l)**
Peptone mix	25.00
D-Sorbitol	10.00
Bile Salts No. 3	1.50
Sodium Chloride	5.00
Neutral Red	0.030
Crystal Violet	0.001
MUG	0.100
Agar	14.50

pH 7.1 +/- 0.2

Directions
Suspend 51g of MacConkey Sorbitol Agar or 51g of MacConkey Sorbitol MUG Agar in 1000ml of cold distilled water; heat to boiling, distribute and sterilise by autoclaving at 121°C for 15 minutes.

Description
MacConkey Sorbitol Agar (SMAC) and MacConkey Sorbitol MUG Agar (SMAC MUG), prepared according to the formulations described by Rappaport and Henig and by Farmer and Davis, are used for the isolation and presumptive identification of *Escherichia coli* O157. *E. coli* O157 produces a toxin which is similar or even identical to that produced by *Shigella dysenteriae* 1, with cytotoxic properties. These strains are characterised by their inability to ferment sorbitol and by their disability to hydrolyse glucuronide derivatives. Both SMAC and SMAC MUC media differ from MacConkey Agar in having sorbitol as a fermentable carbohydrate instead of lactose. SMAC MUG incorporates MUG, to reveal the beta glucuronidase activity. *E. coli* O157 strains do not ferment

sorbitol and grow with colourless colonies and also do not hydrolyse MUG; these colonies are not fluorescent under a Wood's lamp.

Method

1. Inoculate the plates by streaking a suspension of food or faeces onto the surface.
2. Incubate at 37°C for 24 hours. 35-35°C is optimal growth temperature for *E.coli* O157.
3. Observe the plates of SMAC MUG under a Wood's Lamp for the fluorescence emission of the colonies.
4. Do not delay the reading of the plates beyond 24 hours because the colour intensity of sorbitol fermenting strains fade.
5. Confirm the isolated colonies with a slide agglutination test.

E. coli O157 strains do not ferment sorbitol and grow with colourless colonies; these do not hydrolyse MUG and the colonies are not fluorescent under a Wood's lamp on the plates of SMAC MUG.

SMAC Media cannot be used solely to detect VTEC strains of *E.coli* because some *E.coli* O157 strains have atypical appearance on these media and some non-toxic strains do not ferment sorbitol.

Quality assurance (37°C - 24hrs)

Productivity Control
E.coli O157: growth, colourless colonies (not fluorescent under a Wood's lamp)

Specificity Control
E.coli ATCC 25922: growth, red colonies (fluorescent under a Wood's lamp)

Selectivity control
S.aureus ATCC 25923: inhibited

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

References
Centres for Disease Control (1982) Mortal. Weekly Rep. 31, 584.
Doyle, M.P., Schoeni, J. L. (1984) J. Appl. Environ. Microbiol., 48, 855.
Farmer J.J., Davis, B.R. (1985) J. Clin. Microbiol., 22, 620.
Rappaport F and Henig E. (1952) J. Clin. Path. 5,361
Remis, R.S. et al. (1984) Ann. Intern. Med. l01, 624.
Trepeta, R,W., Edberg, S.C. (1984). J. Clin. Microbiol., 19, 172.
Johnson, W.M., Lior, H., Bezanson, G.S. (1983) Lancet, i, 76.

Magnesium Chloride Anhydrous ($MgCl_2$)

Code: SA750

Typical Analysis:

Appearance……White powder
($MgCl_2$)
M.W. 95.21

Precautions:

Do not breathe in dust.

Wear suitable protective clothing, gloves and eye/face protection.

Protect from moisture by keeping container well sealed.

MALONATE BROTH

Used for the differentiation of *Enterobacteriaceae* on the basis of their ability to utilise sodium malonate.

Code: KM3851

Typical formula	**(g/l)**
Amnonium Sulphate	2.00
Potassium Phosphate	1.00
Sodium Chloride	2.00
Sodium Malonate	3.00
Bromothymol Blue	0.025

pH 6.7 + 0.2

Directions
Suspend 8g in 1000ml of cold distilled water, heat to dissolve, distribute into tubes and sterilise by autoclaving at 121°C for 15 minutes. All glassware must be chemically cleaned and alkali-free.

Description
Malonate Broth is used to differentiate *Enterobacteriaceae* on the basis of their ability to utilise sodium malonate as the only source of carbon for their growth. The medium prepared according to the formulation of Leifson, modified by Edwards and Ewing, contains sodium malonate as the only source of carbon and ammonium sulfate as a sole source of nitrogen. Malonate inhibits Krebs cycle by competition with succinic acid, blocking the succinic dehydrogenase activity and glyoxylic cycle by inhibition of isocitratase enzyme, which converts isocitric acid to glyoxylic acid and succinic acid. Sodium malonate inhibits the growth of all the organisms except for those that are able to utilise it as a source of carbon. On the basis of this data Leifson proposed a medium Malonate Broth, for the growth and differentiation of malonate- utilising organisms.

Method
Inoculate the medium with a loopful of pure cultures. Incubate at 37°C for 18-24 hours and then observe the colour change of the medium in tubes. The bacteria, which use sodium malonate as a sole source of carbon, utilise also ammonium sulphate as a source of nitrogen causing an alkalinisation of the medium with a colour change of bromothymol blue from green to Prussian blue. The medium is suitable for the differentiation of *S. arizonae (+)* from *Salmonella spp. (-), Klebsiella - Enterobacter group (+)* from *E.coli (-)* and *Serratia (-)* as well as *Klebsiella (+)* from *Actinobacilllus (-).*

Quality assurance (37°C - 48hrs)

Malonate positive control
K.pneumoniae ATCC 23357

Malonate negative control
E.coli ATCC 25922

Storage
Dehydrated medium: 15-30°C

User prepared tubes: 7 days at 2-8°C

References

Edwards, P.R. & Ewing, W.H. (1972) - Identification of Enterobacteriaceae. 3rd edition, Minneapolis: Burgess Publishing Company.
Leifson, E. (1933) -. J. Bacteriol., 26, 329.
Mac Faddin, J. F. (1976) - Biochemical Tests for Identification of Medical Bacteria. Baltimore: The Williams & Wilkins Company.

MALT EXTRACT AGAR

For the cultivation of yeasts and moulds

Code: KM1059

Typical formula	**(g/l)**
Malt Extract	30.0
Mycological Peptone	5.0
Agar	15.0

pH 5.4 +/- 0.2 (Add Lactic Acid pH will be adjusted to: 3.5 - 4.0.)

MALT EXTRACT BROTH

For the cultivation of yeasts and moulds

Code: KM1060

Typical formula	**(g/l)**
Malt Extract	17.0
Mycological Peptone	3.0

pH 5.4 +/- 0.2

Directions
Suspend 50g of Malt Extract Agar or 20g of Malt Extract Broth in 1000ml of cold distilled water, heat to boiling and autoclave at 115°C for 10 minutes. Prolonged or excessive heating will diminish the gel properties of Malt Extract Agar. To obtain a firmer medium add 5g of agar per litre before sterilisation.

Description
Malt Extract Agar and Malt Extract Broth are used by some laboratories for the cultivation of yeasts and moulds

User quality assurance (25°C -3 days)

Productivity control
C.albicans ATCC 10231: good growth
A.niger ATCC 16404: good growth
P.cyclopium ATCC 16025: good growth
S.cerevisiae ATCC 9763: good growth

Selectivity control
E.coli ATCC 25922: partially inhibited
B,subtilis ATCC 6633:partially inhibited

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

MANNITOL SALT AGAR

A selective medium for the isolation of staphylococci

Code: KM1061

Typical formula	**(g/l)**
Beef Extract	1.00
Peptone mix	10.00
Sodium Chloride	75.00
Mannitol	10.00
Phenol Red	0.025
Agar	12.00

pH 7.4 +/- 0.2

Directions
Suspend 108g in 1000ml of cold distilled water, heat to boiling and sterilise by autoclaving at 121°C for 15 minutes.

Description
Mannitol Salt Agar is a selective medium for the isolation of presumptive pathogenic staphylococci from clinical specimens, milk, meat and foodstuffs. Mannitol Salt Agar is recommended by USP for staphylococci detection in non-sterile pharmaceutical products. The medium prepared according to the formula described by Chapman, contains a high concentration of salt, which inhibits the growth of most bacteria except *Staphylococcus* spp. The production of acids through the fermentation of mannitol, changes the pH of the medium causing a shift of the indicator present in the medium (phenol red) from pink to yellow. Presumptive pathogenic staphylococci, which are coagulase positive grow very well on the medium producing large colonies surrounded by yellow halos. Non-pathogenic staphylococci grow less luxuriously, forming small colonies surrounded by purple halos.

Method
Heavily inoculate the Mannitol Salt Agar plates and incubate at 37°C for 36 hours.
The table below gives the growth characteristics of several microorganisms on Mannitol Salt Agar after incubation of the plates:

Microorganisms	**Growth characteristics**
S. aureus	large yellow colonies surrounded by yellow halos
S.epidermidis	small white colonies surrounded by purple halos
P.vulgaris	completely inhibited
E.coli	completely inhibited
Enterococcus faecalis	partially or completely inhibited
Streptococcus pyogenes	completely inhibited

Quality assurance (37°C - 24/48hrs)

Productivity Control
S.aureus ATCC 25923*: growth, yellow colonies

Specificity Control
S.epidermidis ATCC 12228* growth, pink-violet colonies

Selectivity control
P.mirabilis ATCC 12453*: partially inhibited
*NCCLS M22-A2 recommended strains.

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

References
Chapman, G.H. (1945) - J. Bact. 50, 201-203.
NCCLS document M22-A2, 1996. Quality Assurance for Commercially prepared Microbiological Culture Media-2nd ed.; Approved Standard.
U.S. Pharmacopoeia 24, NF 19 (2000)

MANNITOL SALT BROTH

A selective liquid medium for the isolation of staphylococci

Code: KM6662

Typical formula (g/l)

Beet Extract	1.00
Peptone mix	10.00
Sodium Chloride	75.00
Mannitol	10.00
Phenol Red	0.025

pH 7.4 +/- 0.2

Directions

Suspend 96g in 1000ml of cold distilled water, heat to dissolve, distribute and sterilise by autoclaving at 121°C for 15 minutes.

Description

Mannitol Salt Broth is a selective enrichment medium used for the isolation of presumptive pathogenic staphylococci. The medium contains mannitol as the only fermentable carbohydrate and phenol red as a pH indicator. S. *aureus* show a good growth after 18-48 hours incubation at 37°C with a colour change of the medium from red to yellow.
S. epidermis grow without colour change of the indicator.

User quality assurance (37°C - 36hrs)

Productivity Control
S.aureus ATCC 25923: yellow growth

Specificity Control
S.epidermidis ATCC 12228 pink-violet growth

Selectivity control
P.mirabilis ATCC 12453: partially inhibited

Storage

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

MAXIMUM RECOVERY DILUENT

An isotonic liquid medium for the initial suspension and decimal dilutions of feedstuff for microbiological examination

Code: KM1062

Typical formula (g/l)
Tryptone.....……1.0
Sodium Chloride.....……..8.5

pH 7.0 +/- 0.2

Directions
Suspend 9.5g in 1000ml of cold distilled water and dissolve by heating if necessary. Distribute in 9ml tubes or 90ml flasks and autoclave at 121°C for 15 minutes. Cool to room temperature before the use.

Description
Maximum Recovery Diluent is an isotonic liquid medium for the preparation of initial suspension and decimal dilutions of foods and animal feed stuffs for microbiological examination prepared according to the formulation given by ISO/DIS 6887-1 (Peptone salt).

Method
Prepare in a bowl or plastic bag weigh a mass *m* g or measure a volume *v* ml (minimum 10g or 10ml, unless otherwise stated by specific method) representative of the test sample. The measurement must be +/- 2%.
Add a quantity of Maximum Recovery Diluent equal to 9 x *m* g or 9 x *v* ml. This quantity can be measured by mass with a measurement uncertainty of +/- 2% or by volume with a measurement uncertainty of +/- 5%. It may be necessary in certain cases, particularly for products giving an initial 1+ 9 suspension, which is too viscous or too thick, to add more liquid medium. This should be taken into account for subsequent operations and/or in the resultant expression. If it is necessary, for some enumerations in certain products to fallow below the limit of 10 microorganisms per gram, it is possible to use a smaller volume of diluent. In this case the volume of diluent should be reported in the test report. To avoid damaging the microorganisms the temperature of the diluent during the operation should be approximately the same as the ambient temperature.

Liquid samples: shake the test sample manually by performing 25 up-and down movements of amplitude 30cm in 7s or preferably use a standardised mechanical device to ensure uniform distribution of microorganisms. Take 1ml with a pipette and add this test portion to 9ml of diluent, avoiding contact between the pipette and the diluent. Carefully mix the test portion and the diluent, either by aspirating ten times with a different pipette or in the mechanical mixer for 5 to 10s.

Other samples: operate the peristaltic-type blender for 1-2 minutes according to the nature of the product. Allow the large particle to settle if necessary for up to 15 minutes, then transfer a certain quantity from the

top layer of the suspension to a culture tube, flask or bottle using a large pipette.

Further dilutions: transfer, by means of a fresh pipette, 1ml of the initial suspension (primary 1 + 9 dilution , 10^{-1}) into another tube containing 9ml of sterile Maximum Recovery Diluent, avoiding contact between the pipette and the diluent. Mix carefully, either by aspirating ten times with a fresh pipette or in the mechanical mixer for 5 to 10s, to obtain 10^{-2} dilution. If necessary repeat these operations using the 10^{-2} and further dilutions to obtain 10^{-3}, 10^{-4} etc. dilutions, until the appropriate number of microorganisms has been obtained. Within 30 min. of preparation use the decimal dilutions for inoculating the culture media.

Storage

Dehydrated medium: 15-30°C
User prepared flasks or tubes: 3 months at 2-8°C

References

ISO 6887 Microbiology - General guidance for the preparation of dilutions for microbiological examination. 1983-06-01.
ISO/DIS 6887 – 1 Microbiology of food and animal feeding stuffs- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 1, 1997-10-16.

Meat Peptone (A)

Code: PH1050A

Meat Peptone (A) is produced by the enzymatic digest of selected animal tissue providing a high quality source of peptides. The mixture of peptides present in meat digests is suitable for the growth of most chemoorganotrophic bacteria, especially those of medical importance. It is also commonly used in the preparation of culture media for veterinary products and in the fermentation industry in general.

Description

A yellowish-beige, free-flowing homogenous powder, which produces a clear solution after autoclaving at 121°C for 15 minutes.

Solubility:	2% aqueous
pH:	7.0 ± 0.2

Chemical Characteristics (%) - typical data

α - Amino Nitrogen (AN)	4 - 4.5%
Total Nitrogen (TN)	12.5 - 14%
Loss on drying	3 - 5%
Residue on ignition	15 – 18%

The value given for pH, nitrogen etc. are indicative and many of these values may vary if requested.

Amino Acid Free and linked inpolypeptides (mean values):

AMINO ACID	**Total**
Alanine	5.1%
Arginine	3.9%
Aspartic acid	6.17%
Cystine	---------
Glutamic acid	10.8%
Glycine	7.7%
Histidine	1.5%
Isoleucine	3.14%
Leucine	5.5%
Lysine	5.9%
Methionine	1.5%
Phenylalanine	2.5%
Proline	5.8%
Serine	2.8%
Threonine	2.9%
Tryptohan	0.3%
Tyrosine	0.7%
Valine	3.5%

Storage and Packaging

Store in a cool dry place away from the direct sunlight. Stored in this way in the original packaging, the product has a shelf life of 4 years from date of manufacture.

Available in 500g, 5kg, 10kg, 20 & 30kg sizes, alternative bespoke quantities available upon request. The packaging used is either plastic containers or fibreboard drums (with tamper seal) or corrugated cardboard boxes, depending upon pack size and customer preference.

MEMBRANE LAURYL PEPTONE BROTH

A liquid medium used for the enumeration of coliforms in foodstuffs, water and sewage with MPN technique.

Code: KM1047

Typical formula	**(g/l)**
Tryptone	20.00
Lactose	5.00
Sodium Chloride	5.00
Sodium Lauryl Sulphate	0.10
Dipotassium Hydrogen Phosphate	2.75
Potassium Dihydrogen Phosphate	2.75

Directions
Suspend 35.6g in 1000ml of cold distilled water, heat to dissolve, dispense in quantities of 10ml into tubes containing Durham tubes and autoclave at 121°C for 15 minutes. In the case of double strength suspend 71.2 g in 1000ml of cold distilled water and dispense into tubes of 20x200mm without Durham tubes.
Final pH 6.8 +/- 0.2

Description
Lauryl Peptone Broth is prepared according to Mollan's and Ourby's formula and is recommended by ISO 4831, ICMSF, FDA and APHA as the chosen medium for the presumptive determination of coliforms in foodstuffs, water and sewage.
The sodium lauryl sulphate makes the medium selective for coliforms, whilst the accompanying presence of the phosphate buffer, sodium chloride and the Tryptone allows a faster production of gas even by those bacteria that only slowly ferment lactose. The peptone, phosphate buffer and sodium chloride increase the rhythm of coliform growth. The first during the initial phase of logarithmic growth, the second during the final phase, and the third during the lag phase.

Method
Prepare the test sample and the decimal dilution in accordance with the specific Laboratory method using Maximum Recovery Diluent or other suitable diluent.
Take three tubes of double-strength Lauryl Peptone Broth and by means of a sterile pipette transfer to each tube 10ml of the test sample, if liquid or 10ml of the initial suspension in the case of other products. Than take three tubes of single-strength Lauryl Peptone Broth and by means of a sterile pipette transfer to each tube 1ml of the test sample, if liquid or 10 ml 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 of double-strength Lauryl Peptone Broth at 30 or 37°C for 24 +/- 2 hours. Incubate the tubes of single-strength Lauryl Peptone Broth at 30 or 37°C for 24 +/- 2 hours and for further 24 hours if neither gas nor opacity is observed after 24 hours. From each of the incubated tubes with double-strength Lauryl Peptone Broth inoculate with a loop a tube of Brilliant Green Bile Broth (confirmation medium) and incubate at 30 or 37°C for 24 +/- 2 hours or, if gas formation is not observed, for 48 +/- 2 hours.

Carry out the same procedure for the incubated single-strength Lauryl Peptone Broth showing gas formation or opacity. For each dilution of incubated confirmation liquid medium count the total number of tubes in which gas formation is observed. Express the results as the Most Probable Number of coliforms on the basis of gas production in the Brilliant Green Bile Broth 2% tubes after 48 hours incubation.

Quality assurance (37°C-24 hrs)

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

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
APHA (1985) - Compendium of Methods for Microbiological Examination of Foods, 2nd Ed.
APHA (1985) - Standard Methods for the Examination of Water and Wastewater. 16th Ed.
International IDF Standard 170:1994 - Milk and Milk Products: Enumeration of presumptive *Escherichia coli* content by Most Probable Number Technique, December 1994
FDA (1995) Bacteriological Analytical Manual, 8th ed. Revision A, 1998. Published by AOAC International.
ISO 4831 Microbiology - General guidance for the enumeration of coliforms Most Probable Number Technique, 2nd Ed., 1991-03-01
ISO 7251 Microbiology - General guidance for the enumeration

m - FAECAL COLIFORM AGAR (m-FC AGAR)

A selective medium for isolation and enumeration of faecal coliform organisms

Code: KM3872

Typical formula	**(g/l)**
Tryptose	10.0
Peptone mix	5.0
Yeast Extract	3.0
Sodium Chloride	5.0
Lactose	12.5
Bile Salts No. 3	1.5
Aniline Blue	0.1
Agar	13.0

pH 7.4 +/- 0.2

Directions
Suspend 50g in 1000ml of cold distilled water. Mix well and heat to boiling. Add 10ml of Rosolic Acid 10% solution in NaOH 0.2 N and continue to boil for 1 minute. Do not sterilise in the autoclave. Cool to 50°C and pour into sterile dishes for MF technique (55mm diameter).

Description
m-Faecal Coliform Agar is a selective medium for the isolation and enumeration of faecal coliform organisms with incubation at 44°C according to MF method. The faecal coliform organisms grow with blue colonies; other organisms, whose growth is allowed by the bile salts and incubation temperature, grow with grey colonies.

Method
Filter the sample on a membrane and settle the last over the medium surface.
Tightly close the plate. Incubate in a water-bath at 44°C for 24 hours.
Examine for the presence of blue colonies (faecal coliform organisms)

Quality assurance (44°C-24hr MF)

Productivity control
E.coli ATCC 25922: growth, blue colonies

Selectivity control
E.faecalis ATCC 19433: inhibited

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

m-FAECAL COLIFORM BROTH (m-FC BROTH)

A selective liquid medium for the enumeration of faecal coliform organisms

Code: KM3862

Typical formula	**(g/l)**
Tryptose	10.0
Peptocomplex	5.0
Yeast Extract	3.0
Sodium Chloride	5.0
Lactose	12.5
Bile Salts No. 3	1.5
Aniline Blue	0.1

pH 7.4 +/- 0.1

Directions
Suspend 37g in 1000 ml of cold distilled water, add 10 ml of Rosolic Acid, 10% solution in 0.2 N NaOH. Heat to boiling with agitation, cool and use to saturate sterile absorbent pads for MF technique.

Description
m-Faecal Coliform Broth which is prepared according to the formula proposed by Geldreich, Clark and Bert, is used for the enumeration of the faecal coliform organisms in water by using the membrane filtration method.

Method
Carry out the test by filtering a suitable volume of a water specimen and lay the filters upon absorbent pads imbibed with Faecal Coliform Broth in sterile dishes for MF technique (55mm diameter). Within 30 minutes place in a water-bath at 44-45°C and incubate in airtight containers for 24 hours. The faecal coliform organisms grow with blue colonies whilst the rare colonies formed by non-faecal coliform organisms are cream-grey coloured.

Quality assurance (44°C-24 hrs, MF)

Productivity control
E.coli ATCC 25922: growth, blue colonies

Selectivity control
E.faecalis ATCC 19433: inhibited

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

References
APHA (1985) - Standard Methods of the Examination of Water and Wastewater. 16th edition.
Geldreich, Clark, Huff and Bert (1965) J. Am. Waters Works Ass. 57, Z08.

MICROBIAL CONTENT TEST AGAR

Used to monitor the effectiveness of sanitation procedures

Code: KM6992

Typical formula	**(g/l)**
Tryptone	15.0
Soy Peptone	5.0
Sodium Chloride	5.0
Lecithin	0.7
Polysorbate 80	5.0
Agar	15.0

pH 7.3 +/- 0.2

Directions
Suspend 45.7g in 1000 ml of cold distilled water; heat to boiling with frequent agitation, distribute and sterilise by autoclaving at 121°C for 15 minutes. Cool to 45-50°C and pour into sterile Petri dishes. If it is used with contact plates pout 16.5 to 17.5 ml per plate.

Description
Microbial Content Test Agar is used for the total microbial content test of water-soluble count in cosmetic products and for determining the efficiency of sanitation of containers, equipment and environmental surfaces. The medium contains lecithin and Polysorbate 80, which neutralise the activity of quaternary ammonium compounds and other disinfectants.

MIDDLEBROOK 7H 9 LIQUID MEDIUM

A liquid medium for the isolation, cultivation and identification of the mycobacteria

Code: KM7062

Typical formula	**(g/l)**
Potassium Phosphate Monobasic	1.00
Sodium Phosphate Bibasic	2.50
L-Glutamic Acid	0.50
Sodium Citrate	0.10
Ammonium Sulphate	0.50
Biotin	0.50mg
Calcium Chloride	0.50mg
Copper Sulphate	1.00mg
Zinc Sulphate	1.00mg
Magnesium Sulphate	50.00mg
Fe-Ammonium Citrate	40.00mg
Pyridoxine HCl	1.00mg

pH 6.6 +/- 0.2

Directions
Suspend 4.7g in 900ml of cold distilled water, add 2ml of glycerol and 2g of D-glucose. Heat to dissolve and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and add 100ml of ADC Enrichment filter sterilised. Mix well and distribute into sterile tubes.

ADC Enrichment:
Dissolve 0.24g of oleic acid in 20ml of NaOH 0.05 M (Solution A). Dissolve 4g of bovine albumin (Fraction V) in 200ml of saline solution (let the albumin adsorb, do not mix) – (Solution B). Mix 10ml of Solution A with 190ml of Solution B, heat to 55°C for 10 minutes and sterilise by filtration.

Description
Middlebrook 7H9 Liquid Medium, supplemented as described is recommended in several procedures concerning the isolation, cultivation and identification of mycobacteria, including *Mycobacterium tuberculosis*. Middlebrook 7H9 is recommended by CDC for the preparation of stock culture and for dilutions of the sensitivity test. Middlebrook 7H9 when supplemented with malachite green is suitable for the isolation of the mycobacteria from specimens with mixed flora and for morphological studies. Bovine albumin which acts as protective agent binds free fatty acids that may be toxic to Mycobacterium spp., glucose serves as an energy source, catalase destroy the toxic peroxides that may be present in the medium.

Method
Infectious materials should be submitted to the laboratory without delay and protected from excessive heat and cold. Using aseptic techniques, inoculate a homogenised or centrifuged specimen directly into the liquid medium. Incubate in a 5-10% CO_2 atmosphere at 35-37°C for up to eight weeks. Protect from light or excessive heat as exposure to these elements result in the release of formaldehyde in the medium, which inhibits or kills mycobacteria. Caps of tubes should be loosened for at least one week to allow circulation of CO_2 for the initiation of growth. Caps should be tightened after one week to prevent dehydration of media. Examine the medium within five to seven days and weekly thereafter for up to eight weeks. Turbidity at the bottom or though the tube indicates growth. Mycobacterial growth from the broth can be used for additional laboratory test procedures.

Quality assurance (37°C - up to 21 days CO_2)

Productivity control
M.tuberculosis H37Ra 25177: growth
M.kansaii group 1 12478: growth
M.scrofulaceum Group II 19981: growth
M.intracellulare Group III 13950: growth
M.fortuitum Group IV 6841: growth

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

References
Middlebrook, G. and M.L. Cohn (1958) Am. Rev. Tuberc. 48, 844.
Middlebrook, Cohn, Dye, Russel and Levy (1960) Acta Tuberc. Scand. 38, 66.
Murray et al. Manual of Clinical Microbiology. 6th , ASM.Ed., Washington D.C. 1995

Milk Agar

Used for the enumeration of microorganisms in milk, rinse waters and dairy products.

Code: KM1063

Formulation	**(g/l)**
Yeast Extract	3.0
Peptone	5.0
Skim Milk Powder	1.0
Agar	15.0

pH: 7.2 ± 0.2

Directions
Suspend 24g of powder; disperse in 1 litre of deionised water. Allow to stand for 15 minutes, swirl to mix then sterilise for 15 minutes at 121ºC. Cool to 45ºC before mixing with sample dilutions.

Q.C. organisms: *E. coli , S. aureus*

Storage: Containers for up to 3 months at 15-20ºC in the dark.

Inoculation: Pour plate technique.

Incubation: Aerobically at 30ºC for 72 hours.

References
H.M.S.O., London. British Standard 4285: Methods of Microbiological Examination for Diary Purposes.

MILK PLATE COUNT AGAR

For the enumeration of viable bacteria in milk and dairy products

Code: KM1064

Typical formula (g/l)

Tryptone	5.0
Yeast Extract	2.5
Glucose	1.0
Skim Milk	1.0
Agar	10.0

pH 7.0 ± 0.2

Directions
Suspend 19.5g in 1000ml of cold distilled water. Heat to boiling, stirring until complete dissolution. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Milk Plate Count Agar is used in food microbiology for the enumeration of aerobic bacteria in powdered milk and ice creams, according to the Standards of the International Dairy Federation.

Method
1. Cool the autoclaved medium to 48-50°C.
2. Transfer 1ml of the serial tenfold dilutions of the sample to sterile petri dishes.
3. Pour 15ml of melted medium and mix well with the inoculum.
4. Let the plates solidify and incubate at 30°C for mesophilic bacteria; 55°C for thermophilic bacteria; and at 6.5°C for psychrophylic bacteria.

Quality assurance (30°C – 72hrs)

Productivity control
E.coli ATCC 25922: good growth
S.aureus ATCC 6538: good growth
B.subtilis ATCC 6633: good growth

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

References
FIL-IDF 49:1970 Méthode normalisée pour le denombrement des germes totaux dans les poudres de lait de lactosérum
FIL-IDF 61:1971 Crèmes glacées et glaces au lait: denombrement des germes totaux

MIU (Motility Indole Urea) SEMI-SOLID AGAR

A semi-solid medium for the identification of *Enterobacteriaceae.*

Code: KM5111

Typical formula	**(g/l)**
Tryptone	30.0
Sodium Chloride	5.0
Monopotassium Phosphate	5.0
Phenol Red	0.004
Agar	3.0
Urea	20.0

pH: 6.9 +/- 0.1

Description
MIU semi-solid Agar is used for the differentiation of *Enterobacteriaceae* with motility test, urease and indole production.

Method
Inoculate a test tube by stabbing the medium with a loop that has touched the surface of the centre of a colony. After 18-24 hours of incubation and examine for the following:

Motility: positive (+) diffuse grow around the stabbing line
negative (-) non-diffuse grow around the stabbing line

Urease: positive (+) violet-red colour developing
negative (-) no colour change of the medium

Indole: add 2-3 drops of Kovacs' Reagent to the culture
positive (+) formation of a red-violet ring negative (-) pale yellow colour of the reagent.

MONSUR TTGA BASE AGAR

A selective medium for the isolation of *Vibrio* spp.

Code: KM5211

Typical formula	**(g/l)**
Tryptic Digest of Casein	10.0
Sodium Chloride	10.0
Sodium Taurocholate	5.0
Gelatin	30.0
Sodium Carbonate	1.0
Agar	13.00

pH 8.5 +/- 0.2

Directions
Suspend 69g in 1000ml of cold distilled water. Heat to boiling, distribute and sterilise by autoclaving at 121°C for 15 minutes. Cool to around 50°C and add, aseptically, 0.5ml of Potassium Tellurite 1% Solution. Mix well and pour into sterile petri dishes.

Description
Monsur TTGA Base Agar is a selective medium for the isolation of the organisms belonging to *Vibrio* genus. The presence in the medium of sodium taurocholate and the addition of potassium tellurite inhibit the growth of the most intestinal organisms. *Proteus* only grows, however with small colonies, which are easily distinguishable from the *Vibrio* ones. This medium also inhibits *Pseudomonas* remarkably well.

Method
Inoculate the surface of the agar plate with the specimen or with the specimen enriched in Alkaline Peptone Water. Incubate at 37°C for 24-48 hours. *Vibrio cholerae* on Monsur TTGA has a characteristic morphology. After 24 hours of incubation at 37°C the colonies are transparent and more or less flat, with a grey pigmentation. The centre of the colonies look rather dark if the plates are observed against a white surface. The colonies are often surrounded by a gelatine liquefaction halo. After 48 hours of incubation the colonies are large, with a diameter ranging between 1 to 4mm, transparent with a black centre. The Gelatin liquefaction halo around the colonies becomes definite and clearly visible.

Quality assurance (37°C - 24 hrs)

Productivity control
V.fluvialis NCTC 11212: growth, grey colonies

Selectivity control
E.coli ATCC 25922: inhibited

Storage
Dehydrated medium: 15-30°C
User prepared plates: use the day of preparation

Reference
Monsur, K.A. (1961) Trans. Roy. Soc. Tro. Med. Hyg., 50, 440-442.

MRS AGAR (WITH TWEEN® 80)
Code: KM5071

MRS BROTH (WITH TWEEN® 80)
Code: KM5072

Media used for the cultivation of lactic acid bacteria

Typical formulae	**(g/l)**
MRS AGAR	
Peptone Bacteriological	10.0
Beef Extract	10.0
Yeast Extract	5.0
Glucose	20.0
Dipotassium Hydrogen Phos.	2.0
Sodium Acetate	5.0
Triammonium Citrate	2.0
Magnesium Sulphate	0.2
Manganous Sulphate	0.05
Agar	15.0
Tween® 80	1.0

MRS BROTH	
Peptone Bacteriological	10.0
Beef Extract	10.0
Yeast Extract	5.0
Glucose	20.0
Dipotassium Hydrogen Phos.	2.0
Sodium Acetate	5.0
Triammonium Citrate	2.0
Magnesium Sulphate	0.2
Manganous Sulphate	0.05
Tween® 80	1.0

pH of the medium should be 5.7 +/- 0.1
pH (without adjustment) 6.4 +/- 0.2

Directions
Suspend 70.2g of MRS Agar or 55.2g of MRS Broth in 1000ml of cold distilled water, heat to boiling. Using a pH meter adjust the pH if necessary so that after sterilisation it is 5.7 +/- 0.1 at 25°C. Distribute into bottles of appropriate capacity and sterilise in the autoclave at 121°C for 15 minutes. If there is a risk of extensive yeast contamination of the samples (e.g. dried sausage), add Sorbic Acid to MRS Media as following: dissolve 1.4g of sorbic acid in about 10ml of 1ml/l solution of sodium hydroxide. Sterilise by filtration. Add this solution to 1000ml of MRS Agar, previously cooled to approximately 47°C.

Description
MRS Agar with Tween® 80 and MRS Broth with Tween® 80 prepared according to the formula of De Man, Rogosa and Sharpe, are designed for the cultivation of lactobacilli. Prepared without the tomato extract, the media seem to be particularly sensitive in the isolation of lactobacilli from milk and dairy products. However, lactobacilli of any origin grow on these media. The Tween® 80, sodium acetate and triammonium citrate intensifies the growth of lactobacilli. According to De Man et al. the magnesium sulphate is included in the medium for precautionary measures, since the yeast extract should supply enough magnesium to support the growth of lactobacilli.

Method

For the enumeration of mesophilic lactic acid bacteria in foodstuffs, ISO/DIS 15214 recommends the following technique:

1. Prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerning. ISO 6887 recommending the use of peptone salt (see Maximum Recovery Diluent) as a general diluent for foods and animal feed stuffs.
2. Transfer by means of sterile pipettes 1ml of the test sample (if liquid) or 1ml of the initial suspension and 1ml of each decimal dilution in duplicate to the centre of each empty petri dish.
3. Pour approximately 15ml of MRS Agar with Tween 80, cooled to approximately 47°C into each dish.
4. Mix well with the inoculum the medium and allow the mixture to solidify.
5. Incubate at 30°C for 72-hrs +/- 3 h. Longer incubation may result in excess blackening along the bottom rim of the plates.
6. Count the colonies on the plates containing between 15 and 300 colonies.

If lactic acid bacteria other then mesophilic are to be enumerated, incubate the plates at 42°C for 48hrs (thermophilic lactobacilli) or at 25°C for 5 days (psicrofilic lactobacilli) or at 30°C for 48hrs + 22°C for 48hrs (mesophilic+psicrofilic lactobacilli). Some *Leuconostoc* spp. may form large slimy colonies, which may hinder the development of other colonies, thus causing an underestimation of the number of lactic acid bacteria. *Leuconostoc mesenteroides* and *Leuconostoc dextranicum* are frequently found in the same habitat as lactobacilli, especially *Lactobacillus brevis*, can grow on MRS Agar. These two microorganisms however, can be distinguished by their ability to ferment trehalose, and their inability to hydrolyse arginine. Due to the possible development of microorganisms other than lactic acid bacteria on MRS Agar, it may be necessary in some cases and for some products to confirm the colonies by a simple technique - Gram staining and catalase. MRS Broth may be used as it is for the cultivation of lactic acid bacteria or supplemented with agar, as a solid culture medium for the detection of lactobacilli.

Quality assurance (37°C-48hrs)

Productivity control
L.fermentum ATCC 9338
L.sake ATCC 15521

Storage

Dehydrated medium: 15-30°C
User prepared plates: 15 days at 2-8°C
User prepared flasks or tubes: 1 month at 2-8°C

References

Briggs M. (1953) J. Dairy Res., 20, 36.
Cox, G.P. & Briggs M. (1954) J. App. Bact. 17, 18.
De Man, J.C., Rogosa, M. & Share, M.E. (1960).. J. Appl. Bact . 23, 130-135.
ISO/FDIS 15214 (1998) Microbiolgy of food and animal feeding stuffs – Horizontal method for the enumaration of mesophilic lactic acid bacteria – colony count technique.

MRS AGAR

Selective medium for the isolation and enumeration of lactobacilli

Code: KM1071

Typical formula	**(g/l)**
Peptone mix	6.00
Yeast Extract	9.00
Glucose	10.00
Arabinose	5.00
Sucrose	5.00
Sodium Acetate	15.00
Ammonium Citrate	2.00
Monopotassium Phosphate	6.00
Magnesium Sulphate	0.57
Manganous Sulphate	0.12
Ferrous Sulphate	0.03
Agar	15.00

MRS BROTH

Selective liquid medium for the cultivation of lactobacilli

Code: KM1072

Typical formula	**(g/l)**
Peptone mix	6.00
Yeast Extract	9.00
Glucose	10.00
Arabinose	5.00
Sucrose	5.00
Sodium Acetate	15.00
Ammonium Citrate	2.00
Monopotassium Phosphate	6.00
Magnesium Sulphate	0.57
Manganous Sulphate	0.12
Ferrous Sulphate	0.03

pH 5.4 +/- 0.2

Directions
Suspend 73.7g of MRS Agar or 58.7g of MRS Broth in 1000ml of cold distilled water. Add 1ml of Tween 80 and 1.32ml of acetic acid. Heat to boiling, boil for 2-3 minutes and distribute into sterile containers. The two media do not require sterilisation.

Description
MRS Agar and Broth, prepared according to the formula of Rogosa, Mitchell and Wiseman, are selective media for the isolation and enumeration of lactobacilli from specimens of faecal and oral origin. Rogosa et al. reported that a study comparing MRS Agar and Tomato Juice Agar, using 2000 specimens, showed a good correlation between the two media with regard to the number of lactobacilli found.

MRS Media were found to be more selective in that foreign microorganisms do not grow on them, as they do on Tomato Juice Agar making enumeration of the lactobacilli difficult. MRS Agar was also found to be suitable for the isolation and enumeration of lactobacilli from specimens of vaginal origin.

Quality assurance (37°C-48hrs)

Productivity control
L.fermentum ATCC 9338
L.sake ATCC 15521

Storage
Dehydrated medium: 15-30°C
User prepared plates: 7 days at 2-8°C
User prepared tubes: 1 month at 2-8°C

Reference
Rogosa, M., Mitchell, J.A. & Wiseman R.F. (1971) J. Bact. 62, 132

M.R.V.P. MEDIUM

A medium for the differentiation of Gram-negative enteric bacteria with Methyl Red and Voges-Proskauer tests.

Code: KM1066

Typical formula	**(g/l)**
Peptone mix	7.0
Glucose	5.0
Phosphate Buffer	5.0

pH 7.0 +/- 0.2

Directions
Suspend 17g in 1000ml of cold distilled water, heat to dissolve, distribute and sterilise by autoclaving at 121°C for 15 minutes.

Description
MRVP Medium, developed by Clark and Lubs, is recommended for use in the performance of Voges-Proskauer and Methyl Red tests, as an aid to identify enteric Gram-negative bacilli. The methyl red test is based on the use of a pH indicator to detect acidity when an organism ferments glucose. Because all *Enterobacteriaceae* ferment glucose, acidic metabolic by-products are initially formed. However, with further incubation (2-5 days), *Escherichia coli* and other MR positive bacteria continue to produce more acids. The increased acidity overcomes the phosphate buffer, thus resulting in a low pH and development of a red colour. MR neagative organisms (*Klebsiella pneumoniae and Enterobacter aerogenes*) further metabolise the fermentation products by decarboxylation. The Voges-Proskauer reaction differentiates the microorganisms, which ferment carbohydrates to acids from those that ferment them by decarboxylation to acetylmethylcarbinol (2-8 butyleneglycol fermentation). The acetylmethylcarbinol present in the cultures is oxidised to diacetyl by the atmospheric oxygen in an environment rendered alkaline by the addition of KOH. The diacetyl reacts with the α-naphthol in the solution used for the test giving a pink to red colour. *E. aerogenes* and *K.peneumoniae* cultures give positive test results, *E. coli* negative.

Method
Using organisms taken from 18-24 hour pure culture, lightly inoculate the MR-VP Medium. Incubate aerobically at 35-37°C for 24 hours. After 24 hours of incubation transfer 3ml of broth to a clean test tube. Re-incubate the remaining broth for an additional 24 hours.

Voges-Proskauer Test:
Prepare the following solutions: Creatine monohydrate: dissolve 0.5g in 100ml of distilled water 5% α-naphthol: suspend 6g of α-naphthol in 100ml of ethyl alcohol 96%. Prepare a fresh solution for the test. 40% KOH: suspend 40g of KOH in 100ml of distilled water. To 3ml of broth culture add 2 drops of creatine solution 3 drops of α-naphthol solution and 2 drops of KOH solution. Gently shake the tube to expose the medium to oxygen. A positive test is indicated by the development of a pink to bright red colouration 15 minutes after the addition of the reagents.

Methyl red Test:
After 5 days of incubation, transfer 5ml of broth to a clean test tube. Add 5 drops of methyl red prepared as follows: suspend 0.1g of methyl red in 300ml of 95% ethyl alcohol, and make up to 500ml with distilled water. The reaction is positive if a red colouration develops immediately. A negative reaction is indicated by a yellow colour on the surface of the liquid medium.

Quality assurance (37°C - 24hrs)

MR Positive, VP negative control
E.coli ATCC 25922

MR negative, VP positive control
K.pneumoniae ATCC 23357

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

References
AFNOR V08-052 Methode de routine pour la recherché de Salmonella. Septembre, 1993
Barn, M.M. (1986), The intensification of the Voges-Proskauer reaction by the addition of α-naphthol. J. Pathot. Bacteriol., 42, 441
Edwards, P.R. & Ewing. V.H. (1965). Identification of *Enterobacteriaceae.* Minneapolis: Burgess Publishing Company.

MSE AGAR

For the enumeration of *Leuconostoc* in milk, dairy products and sweet foods

Code: KM3381

Typical formula (g/l)

Tryptone	10.00
Gelatin	2.50
Yeast Extract	5.00
Sucrose	100.00
Glucose	5.00
Sodium Citrate	1.00
Sodium Azide	0.075
Agar	13.00

pH 6.9 ± 0.2

Directions

Suspend 136.5g in 1000ml of cold distilled water. Heat to boiling, stirring constantly and autoclave at 110°C for 15 minutes.

Description

MSE Agar is prepared according to the typical formulation developed by Meyeux, Sandine and Elliker. It is used for the detection and enumeration of *Leuconostoc* in milk, dairy products and sweet foods. Gram-negative bacteria are inhibited by sodium azide present in the medium. Lactic streptococci are capable of growing with small opaque white or yellowish colonies after 4 days.

Method

1. Inoculate the surface of the medium with 0.1ml of the sample and its tenfold dilutions and spread the inoculum.
2. Incubate at 21°C and examine daily for 4 days
3. *L. mesenteroides* and *L.dextranicum* will grow with gelatinous colonies because they metabolise the glucose with the production of dextrans.

Storage

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

References

Majeux, J.V., Sandine, W.E., Elliker, P.R. (1962) J. Dairy Science, 45, 655

MSRV SEMI-SOLID MEDIUM

A medium for the detection of motile strains of *Salmonella* in foodstuff, environmental specimens and chocolate products

Code: KM1099

Typical formula	**(g/l)**
Tryptose	4.59
Acid Digest of Casein	4.59
Sodium Chloride	7.34
Monopotassium Phosphate	1.47
Magnesium Chloride Anhydrous	10.93
Malachite Green Oxalate	0.037
Agar	2.70

pH 5.2 ± 0.1

Directions
Suspend 15.8g of MSRV Medium in 500ml of cold distilled water. Heat to boiling with agitation. **Do not** autoclave this medium. Cool to approximately 50°C and add, under aseptic conditions, the contents of one vial of Novobiocin Supplement, reconstituted with 5ml of sterile distilled water. Mix well and distribute into sterile petri dishes and leave to dry for one hour. The base medium is very hygroscopic. Keep the bottle lid very tight in an air-dry room. Handle the powder wearing a mask and gloves.

Description
The MSRV medium supplemented with Novobiocin supplement allows the detection of *Salmonella* in foodstuff. The use of this medium, prepared according to the typical formulation of De Smedt et al. (1986), attains better results in the isolation of *Salmonella* (De Smedt and coll. 1991, Perales and coll. 1991), with respect to usual methods. The principle is based on the ability of salmonellae to grow and migrate in a semi-solid gel from the inoculation point, quicker than other competitive microorganisms do, producing in this way an enrichment and a differentiation between samples inducing the migration and samples not inducing this phenomenon. This medium is not suitable for the detection of non- motile strains of *Salmonella,* those of which their presence is very low (lower than 0.1%). Should the presence of non-motile strains be suspected, it is suggested to follow a traditional procedure with a pre-enrichment and selective enrichment. The medium selectivity is due to the presence of three antimicrobials: malachite green, magnesium chloride and novobiocin.

Method
1. Carry out the pre-enrichment with Buffered Peptone Water or Skim Milk supplemented with 2ml/ltr of Brilliant Green 1% solution (25g of sample + 225ml of pre-enrichment broth).
2. After 20hrs incubation at 37°C transfer three drops (approximately 0.1ml) on a MSRV plate medium. Incubate at 42°C for 24hrs in a non-inverted position.
3. Transfer 1ml of the incubated pre-enrichment culture in 10ml of Selenite Cystine Broth and incubate at 37°C; also transfer 0.1ml in 10ml of Rappaport Vassiliadis Medium and incubate at 42°C.

4. After 8hrs of incubation, transfer three drops of both selective broths on plates of MSRV Medium; incubate for 16hrs at 42°C.
5. Check the MSRV Medium plates for migration halos.
6. Remove the growth from the border of the migration area, for serological and biochemical confirmation tests.
7. For more details of the analytical procedure, refer to the paper of De Smedt and Bolkerdijk: Collaborative Study of the International Office of Cocoa and Chocolate, and Sugar Confectionery on the use of Motility Enrichment of *Salmonella* detection in cocoa and chocolate.

Quality assurance (42°C-16hrs)

Productivity Control
S.typhimurium ATCC 14028: growth surrounded by halo

Selectivity control
C.freundii ATCC 8090: restricted or no growth

Storage
Dehydrated medium: 15-30°C
User prepared plates: 5 days at 2-8°C

References
De Smedt, J.M. and Bolderdijk, R.F. (1990) J. Food Proct. 53, 659
De Smedt, J.M. et al. (1986) J. Food Proct. 49, 510-514
IOCC/ISCM, Analytical Method 118, 1990. Microbiological Examination of Chocolate and other Cocoa Products; Draft Standard Method.
De Smedt, J.M. et al. (1991) Int. J. Food Microbiol. 13, 301-308
Perales, I. and Erkiaga, E. (1991) Int. J. Food Microbiol. 14, 51-58

MUCAP TEST

An Enzymatic fluorescent test for the immediate identification of *Salmonella* colonies by C_8 esterase detection on enteric plating media

Code: 191500

Pack contents
4 methylumbellyferil caprilate dissolved in an organic solvent 8 ml

Warning: the reagent is inflammable. Do not expose close to heat sources.

Description
MUCAP Test is an easy and rapid method that allows the presumptive detection of *Salmonella* spp. on plating media. It contains a substrate conjugated with methylumbelliferone, cleaved by C_8 esterase enzyme of *Salmonella* spp. releasing a strongly fluorescent compound. Several authors have studied the performance of the MUCAP Test in order to evaluate its sensitivity and specificity. The data published show that the sensitivity of *Salmonella* detection with MUCAP test is always near to 100% and the specificity is more variable depending on the isolation media and/or the combination of enrichment and isolation media.

The relevant published data is as follows: Ruiz, J., et al. (1992) sens. 100%, spec. 99.8%; Olsson M., et al.: sens. 100%, spec. 99.2%; Ruiz J., et al. (1991): sens. 100%, spec. 92.4%, (98.2% when the oxidase test is used together with MUCAP test; Aguirre, P.M., et al: sens. 95%, spec. 88-95%; Munoz P., et al.: sens. 100%, spec. 91%, Anichini P., et al.: sens. 100%, spec. 92.3%; Agostinelli A., et al.: sens. 100%, spec. 97%, Manafi M., et al.: sens. 100%, spec. 80%.

Technique
The test is carried-out on a primary isolation medium by flooding with one drop of MUCAP reagent of all the suspect colonies (H_2S positive and/or lactose negative colonies). The following scheme should be used: Observe the colonies under a Wood's lamp (366nm) before adding the reagent to ensure that no spontaneous fluorescence occurs. Add a drop of reagent to each isolated colony or to a group of colonies After 3-5minutes observe the plates under the Wood's lamp (wavelength 366nm) in semi-darkness.

Positive result: Appearance of a blue fluorescence over the whole colony or on the edge of black centred colonies.

Negative result: No development of fluorescence

The fluorescent colonies can be identified presumptively as *Salmonella* spp. and subjected to a complete identification with biochemical and serological tests.
The fluorescent negative colonies can be considered not belonging to the *Salmonella* genus and so plates are rejected.

Limitations
- As *P. aeruginosa* is often responsible for false positive results, the H_2S negative and fluorescent positive colonies can be tested for the oxidase test. The H_2S negative MUCAP positive and oxidase negative colonies can be identified as *Salmonella* spp. without waiting for the final result in case where an immediate diagnosis is needed.
- The reagent does not affect the viability of the flooded colonies.

- Do not take any reading after 5minutes because the reagent is susceptible to autolysis due to the watery nature of the medium and so false positive results might occur.
- The MUCAP Test can be carried-out on must culture media normally used i.e. MacConkey, SS, HEA, DCA, XLD, etc. - Exceptions: Bismuth Sulphite Agar.

References

Agostinelli, A., Agostinelli, C., Damiani, A.M., Gambella, O., (1990. Microbiologia Medica. Vol. 5 n° 3, 129
Aguirre, P.M., Cacho, J.B., Folgueira, L., Lopez, M., Garcia, J., Velasco, A.C. (1990) J. Clin. Microbiol. 28, 148
Anichini, P., Lavacchi, A., Pecile, P., Nicoletti, P., (1991) Microbiologia Medica Vol. 6, n° 4, 117
Malfa, S., Potenza, D., (1986). XV Congresso Nazionale AMCLI, Milano
Manafi, M., Sommer, R., (1992) Lett. App. Microbiol. 14, 163
Munoz, P., Diaz, D., Carcemado, E., Rodriguez, M., Rodeno, P., Bonza, E., (1990) 30th ICAAC, Atlanta, Ga
Olsson., M., Syk, A., Wollin, R., (1991). Clin. Microbiol. 29, 2631
Pontello, M., Russolo, S., Carozzi, F., Bottiroli, U., (1987) 5th Int. Simp. on Rapid Method and Aut. in Microb. and Immunol. Florence, 4-6 nov. 1987
Ruiz, J., Varela, M.C., Sempere, M.A., Lopez, M.L., Gomez, J., Oliva, J., (1991) Eur. J. Clin. Microbiol. Infect. Dis. 10, 649
Ruiz, J., Sempere, M.A., Varela, M.C., Gomez, J., (1992) J. Clin. Microbiol. 30, 525
Russolo, S., (1986) Università degli Studi di Milano.

MUELLER HINTON AGAR II

An improved powdered antimicrobial susceptibility testing medium

Code: KM1067

Typical formula	**(g/l)**
Beet Extract	2.0
Acid Digest of Casein	17.5
Starch	1.5
Agar Bios Special	17.0

pH 7.3 +/- 0.1

Directions
Suspend 38g in 1000ml of cold distilled water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes.

Description
Mueller Hinton Agar was originally prepared for the cultivation of gonococci and because of its low level of p-aminobenzoic acid it was used for sensitivity tests with sulphonamides. Mueller Hinton Agar is recommended by NCCLS (Document M2-A4, Approved Standard) for antimicrobial disc susceptibility test of most common, rapidly growing bacteria, by the Kirby-Bauer diffusion method. To test strains that fail to grow satisfactorily on Mueller Hinton Agar, 5% defibrinated sheep blood is added to the melted and cooled medium. For tests with *H. influenzae* the Mueller Hinton Agar must be supplemented with l5mg/L NAD, 15mg/L bovine hematin, 5g/ltr yeast extract (see Haemophilus Test Agar). Mueller Hinton Agar II is prepared with selected raw materials. It contains low levels of thymine and thymidine since they affect sulphonamide and trimethoprim MIC values and controlled levels of Ca++ and Mg++ to obtain aminoglycosides inhibition zones against *P. aereuginosa* and tetracycline inhibition zones against staphylococci within the ranges suggested by NCCLS M2-A4.

Method
Prepare Mueller Hinton Agar II in plates of 100mm or 140mm with a medium layers of 4mm (25ml of medium per plate of 9cm and 60ml per plate of 14cm). To prepare an inoculum, suspend 4-5 colonies grown in a primary isolation medium in 4-5ml of Tryptic Soy Broth and incubate for 2-6 hours until the broth culture reaches the same density as the opacimetric standard prepared by adding 0.5ml of .1% barium chloride to 99.5ml of 0.36 N sulphuric acid. Within 15 minutes, dip a sterile swab into the broth culture, squeeze it against the walls of the tube to remove excess liquid, and then streak it over the surface of the agar plates to obtain a uniform distribution of the inoculum. Leave the plates to dry then lay the paper discs pressing them onto the surface of the agar. Incubate at 35°C for 18 hours then read the inhibition zones by taking in to consideration the zones, which are completely free of microbial growth and which have distinct borders. Compare the zone sizes obtained to those reported on the table no. 2 of the NCCLS Document M2-A4 "Zone Diameter Interpretative Standards" to detect whether the organism is resistant, intermediate or susceptible to antimicrobial agents.

Quality assurance (37°C-24 hrs)

<u>Mueller Hinton Agar II</u>
S.aureus ATCC 25923
E.coli ATCC 25922
P.aeruginosa ATCC 27853
E.faecalis ATCC 33186

<u>Mueller Hinton Blood Agar</u>
S. pnoumoniae ATCC 6805.
Muller Hinton Haemophilus Test Medium
H. influenzae ATCC 10211

Inhibition zones within the ranges recommended by NCCLS

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

References
Bauer, A.W.; Kirby, W.M,M,; Sherris, K.C. & Truck, M. (1966), Amer. Clin. Path, 45, 493-496.
National Committee for Clinical Laboratory Standard. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard M2 A4 - Seventh Edition. American National Standard.

MUELLER HINTON BROTH

A susceptibility testing liquid medium

Code: KM1068

Typical formula	**(g/l)**
Beef Extract	3.0
Acid Digest of Casein	17.5
Soluble Starch	1.5

pH 7.3 +/- 0.2

Directions
Suspend 22g in 1000ml of cold distilled water, heat to dissolve, distribute and sterilise by autoclaving at 121°C for 10 minutes.

Description
Mueller Hinton Broth was originally prepared for the cultivation of gonococci, and because of its low level of p-amino-benzoic acid, was used for sensitivity tests on sulphonamides. It is recommended by NCCLS Document M7-A2, Approved Standard: "Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically" for testing the susceptibility of *H. influenzae,* Mueller Hinton Broth must be supplemented with bovine hematin, NAD, yeast extract, thymidine phosphorilase. For reference methods concerning the determination of minimum inhibitory concentrations of aerobic bacteria by broth macroditution, broth microdilution and agar dilution, refer to NCCLS Approved Standard.

Storage
Dehydrated medium: 15-30°C
User prepared tubes: 3 months at 2-8°C

Reference
National Committee for Clinical Laboratory Standard. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard M7-A2 —Fifth Edition

MULLER KAUFFMAN TETRATHIONATE BROTH BASE

An enrichment broth base for the enrichment of *Salmonella* spp. in foodstuff and other specimens.

Code: KM1069

Typical formula	**(g/l)**
Tryptone	7.00
Soy Peptone	2.30
Sodium Chloride	2.30
Calcium Carbonate	25.00
Sodium Thiosulphate	40.70
Bile Salts	4.75

Directions
Suspend 82g in 1000ml of cold distilled water. Heat to boiling and cool to below 45°C. Add 19ml of iodine solution and 9.5ml of a 0.1% brilliant green solution. Mix well and distribute into sterile tubes or flasks. Do not heat after the iodine solution has been added.

Iodine solution:

Iodine	20g
Potassium Iodide	25g
Distilled Water	100ml

Dissolve the potassium iodide in 5ml of distilled water, add the iodine and gently warm the solution to dissolve completely. Make the volume up to 100ml with distilled water.

Brilliant green solution:

Brilliant green	0.1g
Distilled water	100.0ml

Add the brilliant green to the distilled water, shake and heat at 100°C for 30 minutes to ensure that the dye has completely dissolved. Store in a brown bottles.

Description
Muller Kauffmann Tetrathionate Broth, developed by Muller (1923) was later modified by Kauffmann with the addition of brilliant green to inhibit the growth of *Proteus* spp., and bile salts to increase the recovery of salmonellae. The medium can also be supplemented with novobiocin 4mg/Ltr to improve the inhibition of *Proteus* spp. Muller Kauffmann Tetrathionate Broth is recommended for enrichment of *Salmonella* spp. in food, water, milk, ice cream and pasteurised egg-base products. The medium is usually used together with Selenite Cystine Broth or Rappaport Vassiliadis Soy (RV) Broth and this selective enrichment is preceded by a non-selective enrichment in Buffered Peptone Water. The recommended incubation temperature of the selective enrichment with Muller Kauffmann Tetrathionate Broth is 43°C. This liquid enrichment medium should not be used if *Salmonella typhi* is suspected.

Method

1. Transfer two aliquots of 10ml of the pre-enriched culture in Buffered Peptone Water to 100ml of Muller Kauffmann Tetrathionate Broth and to 100ml of Selenite Cystine Broth.
2. Incubate the inoculated Muller Kauffmann Tetrathionate Broth flasks at 43°C for 24 hours.
3. Incubate the inoculated Selenite Cystine Broth flasks at 37°C for 24 hours.
4. Using the cultures obtained in Muller Kauffmann Tetrathionate Broth and Selenite Cystine Broth inoculate by means of a 3mm loop, two large-size petri dishes or four 90mm petri dishes containing Brilliant Green Agar Modified.
5. Proceed in the same way with a second plating medium – e.g. Chromogenic Salmonella Agar, or other suitable selective *Salmonella* plating-out medium chosen by the laboratory.

Quality assurance (43°C-24hrs, subculture on TSA)

Productivity Control
S.typhimurium ATCC 14028: growth

Selectivity control
E.coli ATCC 25922: partially or completely inhibited

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

MYCOLOGICAL AGAR

For the cultivation and maintenance of fungi

Code: KM1070

Typical formula (g/l)

Soy Peptone	10.0
Glucose	10.0
Agar	15.0

pH 7.0 +/- 0.2

Directions

Suspend 35g in 1000ml of cold distilled water, heat to boiling and sterilise by autoclaving at 115°C for 15 minutes. Cool and use at once. **Do not** re-melt or overheat at any time.

Description

Mycological Agar is a general purposes medium for the cultivation and maintenance of fungi. Mycological Agar may be used for the examination of clinical specimens, often in parallel with selective media. The medium may be used at pH 4.0 by the addition of 15ml/l of 10% lactic acid for the enumeration of yeasts and moulds in carbonated beverages, beer, sugar and other materials.

Quality assurance (25-30°C, up to 7 days)

Productivity Control

C.albicans ATCC 60193: growth

T.mentagrophytes ATCC 9533: growth

Storage

Dehydrated medium: 15-30°C

User prepared plates: 1month at 2-8°C

MYCOLOGICAL SELECTIVE AGAR

A selective medium for the isolation of pathogenic fungi.

Code: KM1070S

Typical formula	**(g/l)**
Soy Peptone	10.00
Glucose	10.00
Cycloheximide	0.40
Chloramphenicol	0.05
Agar	15.00

pH 7.0 +/- 0.2

Directions
Suspend 35.5g in 1000ml of cold distilled water, heat to boiling and sterilise by autoclaving at 118°C for 15 minutes. Cool and use at once. **Do not re-melt or overheat at any time**.

WARNING: Cycloheximide is extremely toxic and causes severe irritation of the skin and mucous membranes. **Use with care!**

Description
Mycological Selective Agar is used for the isolation of pathogenic fungi from specimens with mixed flora. The medium incorporates a nutritive base, which supports good growth of both bacteria and fungi. The neutral pH permits cultivation of pathogenic fungi, which cannot grow on other media such as Sabouraud Maltose Agar because of the acid pH. The incorporation of two antibiotics; chloramphenicol and cycloheximide, make the medium selective. The chloramphenicol inhibits bacterial growth, cycloheximide the growth of saprophytic fungi, which is far more sensitive to the action of antibiotics than pathogenic fungi. Mycological Selective Agar is used for the isolation of both dermatophytes and fungi, which prove systemic infections. Cycloheximide also inhibits the growth of certain pathogenic fungi, such as *Cryptococcus neoformans.* The incubation temperature may modify the sensitivity of certain pathogenic fungi due to the antibiotics in the medium. For this reason, it is possible to use Mycological Selective Agar alone in the isolation of dermatophytes. Whereas, for the isolation of fungi responsible for systemic infections it is advisable to use Mycological Selective Agar together with other media, which do not contain antibiotics. Additionally, incubation should take place at room temperature and at 37°C.

Quality assurance (25°C- up to 7 days)

Productivity control
T.mentagrophytes ATCC 9533*: growth, the medium turns red-violet
C.albicans ATCC 10231*: growth

Selectivity control
E.coli ATCC 25922*: inhibited
A.niger ATCC 16404*: inhibited
*NCCLS M22-A2 recommended strains.

Storage
Dehydrated medium: 15-30°C

User prepared plates: 1 month at 2-8°C

References
Booth, C. (1971) - Methods in Microbiology, Vol. 4, London: Academic Press.
NCCLS document M22-A2, 1996. Quality Assurance for Commercially prepared Microbiological Culture Media-2nd ed.; Approved Standard.
Stein, R.J. (1973) - Handbo of Phycological Methods, Cambridge: University Press.

M17 AGAR

A selective medium for the enumeration of *Streptococcus thermophilus* from yoghurt and for improved growth of lactic streptococci and their bacteriophages

Code: KM1051

Typical formula	**(g/l)**
Tryptone	2.50
Peptone	2.50
Soy Peptone	5.00
Yeast Extract	2.50
Beef Extract	5.00
Sodium Glycerophosphate	19.00
Magnesium Sulphate	0.25
Ascorbic Acid	0.50
Lactose	5.00
Agar	13.00

pH 7.2 +/- 0.2

Directions
Suspend 55.2g in 1000ml of cold distilled water. Heat to boiling, distribute and sterilise by autoclaving at 121°C for 15 minutes.

Description
M17 Agar is used for the isolation and the enumeration of the lactic streptococci from yoghurt, cheese, starter cultures and other dairy products. The medium is recommended by International Dairy Federation, for the selective isolation and enumeration of *S. thermophilus* in yoghurt. The high concentration of sodium glycerophosphate inhibits the growth of *L. bulgaricus* while the high buffering power allows the cultivation of lactic streptococci. M17 Agar is prepared according to the formulation described by the IDF. It recommends M17 Agar also for the demonstration of lactic bacteriophage activity. When this method is see the relevant reference: Appl. Microbiol 29,807, 1975), 100ml of medium is supplemented with 10ml $CaCl.6H_2O$ 1.0 M.

Method
The enumeration of *S. thermophilus* in yoghurt is carried out according to the following work procedure:

Mix the contents of the yoghurt pot by using a sterile spatula. In the case of fruit-yoghurt homogenise the contents of the pot for one minute.
Weigh 10g of product in an appropriate 200ml bottle and bring to 50g with an autoclaved peptone solution having the following composition:

Tryptone	0.5g
Peptone	0.5g
Distilled water	1000ml

Homogenise for 1 minute. Bring to 100g by using the same diluent a 1:10 dilution being this obtained. Prepare a suitable series of decimal dilutions of the sample suspension in 9ml of 0.1% peptone solution. From each tube, pipette 1ml of the appropriate dilution in a 90 or 100mm petri dish in duplicate. Add 14ml of Ml7 Agar, cooled to 43°C +/-1 to each dish. Mix and leave to solidify. Incubate for two days at 35°C for 48 hours.
Examine the plates after 24 and 48 hours incubation. Under these conditions *S. thermophilus* grows with lenticulate appraisable colonies after 18-24 hours and reach 1-2mm diameter within 48 hours. *L. bulgaricus* may grow in the form of very small colonies. Carry out the enumeration in each petri dish that does not contain more than 400 colonies and express the result taking the dilution factor into account as the number of colony forming units per gram of specimen. From each dish considered suitable for the expression of results, take up a number of colonies correspondent to

the square root of the total number examined. Lie on a slide and perform a Gram stain to verify that these are Gram-positive cocci. Perform catalase test (negative).

Quality assurance (37°C - 48hrs)

Productivity Control
S.thermophilus ATCC 14485: growth

Selectivity control
L.bulgaricus ATCC 11842: partially or completely inhibited

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

References
International Dairy Federation (1981) Joint IDF/1 SO/AOAC Group E 44

M 17 BROTH

For improved growth of lactic streptococci and their bacteriophages

Code: KM1052

Typical formula	**(g/l)**
Tryptone	2.50
Peptone	2.50
Soy Peptone	5.00
Yeast Extract	2.50
Beef Extract	5.00
Sodium Glycerophosphate	19.00
Magnesium Sulphate	0.25
Ascorbic Acid	0.50
Lactose	5.00

pH 7.2 +/- 0.2

Directions
Suspend 42.2g of powder in 1000ml of cold distilled water. Heat to boiling, distribute and sterilise by autoclaving at 121°C for 15 minutes.

Description
M17 Broth is suitable liquid medium for the maintenance of starter cultures. It is used in conjunction with M17 in bacteriophage assay.

Quality assurance (37°C - 48hr)
Productivity Control
S.thermophilus ATCC 14485: growth

Selectivity control
L.bulgaricus ATCC 11842: partially or completely inhibited

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

M.L.C.B. Agar (Mannitol, Lysine, Crystal Violet, Brilliant Green Agar**)**

A selective medium for the isolation of Salmonella spp. (with the exception of S. typhi and S. paratyphi A) from food and faeces. Salmonella colonies are recognised by distinctive colonial appearance and H2S production and this medium will also detect lactose and sucrose fermenting strains.

Code: KM1065

Typical formula	**(g/l)**
Yeast Extract	5.0
Tryptone	5.0
Meat Peptones	7.0
Sodium chloride	4.0
Mannitol	3.0
L-Lysine HCL	5.0
Sodium thiosulphate	4.0
Ferric ammon. citrate	1.0
Brilliant green	0.012
Crystal violet	0.01
Agar	15.0

pH: 6.8 ± 0.2

Directions
Weigh 49 grams of powder, disperse in 1 litre of deionised water. Allow to stand for 10 minutes, swirl to mix then bring to the boil with frequent agitation to completely dissolve the powder. Cool to 47ºC and pour plates. DO NOT AUTOCLAVE OR OVERHEAT.

CAUTION: Some problems may occur with H2S negative strains, eg S. pullorum, S. senftenberg, S. sendai and S. berta. This medium should not be used to detect S. typhi and S. paratyphi A, as these strains are more susceptible to the brilliant green dye.

Q.C. organisms: *Salmonella spp. , E. coli* (inhibition)

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

Inoculation: Surface plating, streaking for single colonies. Inoculation can be carried out directly, or from enrichment broths. Because of the low selectivity of this medium the inoculum should not be heavy, and it is recommended that this medium should be used in conjunction with other more selective media.

Incubation: 37ºC aerobically for 24 hours.

MacCONKEY AGAR MUG

A medium for the rapid detection of *E.coli* in urine samples and other specimens

Code: KM6722

Typical formula	**(g/l)**
Peptone	17.00
Peptone mix	3.00
Lactose	10.00
Bile Salts No.3	1.50
Sodium Chloride	5.00
Neutral Red	0.030
Crystal Violet	0.001
Agar	13.500
MUG	0.1

pH 7.1 +/- 0.2

Directions
Suspend 50g in 1000ml of cold distilled water, heat to boiling and sterilise by autoclaving at 121ºC for 15 minutes. Dry the surface of the medium before inoculation.

Description
MacConkey Agar MUG is used for the enumeration of *Entero-bacteriaceae* and for the rapid detection of *E. coli* in urine samples or other specimens by means of the detection of the enzyme β-glucuronidase.

Method
Incubate the streaked plates for 18-24 hours at 37°C. The split of 4-methylumbelliferyl component is fluorescent at 366nm. Report as *E. coli* the colonies with a fluorescence development under a Wood's lamp. Because the *E. colt* is the most common pathogen found in urine, the rapid method with MacConkey Agar MUG allows to save time and work in the microbiological examination of urines.

Quality assurance (37°C - 24hrs)

Productivity Control
E.coli ATCC 25922: growth, red purple colonies, fluorescent under a Wood's lamp

Specificity Control
E.aerogens ATCC 13048: growth, red purple colonies, no fluorescent under a Wood's lamp

Selectivity control
S.aureus ATCC 25923: inhibited

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

Reference
Trepeta, A. and Edberg, S.C. (1984) J.Clin. Microbiol. 19, 172-174

MacConkey Agar No. 3 (With Crystal Violet)

Association for the isolation of Enterobacteriaceae from waters and sewage.

Code: KM1056

Formula	**(g/l)**
Peptone	20.0
Lactose	10.0
Bile Salts No. 3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0

pH: 7.1 ± 0.2

Directions:
Weigh 51.5 grams of powder and add to 1 litre of deionised water. Allow to stand for 10 minutes, swirl to mix then sterilise for 15 minutes at 121ûC. Cool to 47ûC and pour into petri dishes. Dry the surface before inoculation.

Description
A modification recommended by the W.H.O. and the American Public Health Association for the isolation of Enterobacteriaceae from waters and sewage. The medium has been made more selective than MacConkey's original formula by the use of crystal violet as well as bile salts. Gram-positive organisms will not grow on this medium.

Q.C. organisms: *E. coli, Ent. faecalis* (inhibition)

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

Inoculation: Surface, streaking for single colonies.

Incubation: 37ºC aerobically for 18-24 hours.

References
American Public Health Association 1950. Diagnostic Procedures and Reagents. 3rd edn. A.P.H.A., New York. American Public Health Association 1946. Standard Methods for the examination of Water and Sewage. 9th edn. A.P.H.A., New York.

MacCONKEY AGAR without SALT

A medium for the isolation and differentiation of lactose and non-lactose fermenting enteric bacteria. Useful in a wide range of clinical material and has applications in food, water and dairy bacteriology.

Code: KM1054

Typical formula	**(g/l)**
Peptone	20.00
Lactose	10.00
Bile Salts	5.00
Neutral Red	0.05
Agar	15.00

pH 7.4 +/- 0.2

Directions
Suspend 50g in 1000ml of cold distilled water, heat to boiling and autoclave at 121°C for 15 minutes. Dry the surface of the medium before inoculation.

Description
MacConkey Agar W/O Salt, has the same formula as other MacConkey Agars with the omission of sodium chloride. The medium has low electrolyte content and, therefore, the *Proteus* spp. do not spread. The medium is recommended for the bacteriological examination of urine because *Proteus* overgrowth is prevented.

Quality assurance (37°C - 24hrs)

Productivity Control
E.coli ATCC 25922: growth, red pink colonies
P.mirabilis ATCC 25933: growth, colourless colonies, no swarming

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

MACCONKEY AGAR (WITH SALT)

A differential and moderately selective medium, for the isolation and differentiation of coliform bacteria and intestinal pathogens in water, dairy products and other specimens.

Code: KM1053

Typical formula	**(g/l)**
Peptone mix	20.00
Lactose	10.00
Bile Salts	5.00
Sodium Chloride	5.00
Neutral Red	0.05
Agar	12.00

pH 7.4 +/- 0.2

Directions
Suspend 52g in 1000ml of cold distilled water, heat to boiling and autoclave at 121°C for 15 minutes. Dry the surface of the medium before inoculation.

Description
MacConkey Agar is a differential and moderately selective medium for the isolation and differentiation of coliform bacteria and intestinal pathogens in water, dairy products and other specimens. MacConkey Agar corresponds to the medium recommended by the WHO, The Dept. of Health, and by Windle Taylor for the isolation of coliforms in water. The medium is slightly less selective than MacConkey Agar, because of the omission of crystal violet and the use of bile salts instead of Bile Salts No.3. On MacConkey Agar the coliforms produce red-violet colonies after an incubation of 18-24 hours at 37°C. The enteric bacteria that do not ferment lactose produce transparent or opaque yellow colonies.

Microorganisms	**Growth characteristics**
Escherichia coli	from red to pink colonies
Enterobacter aerogenes	mucoid pink colonies
Salmonella	transparent colourless colonies
Shigella	transparent colourless colonies
Pseudomonas	transparent colourless colonies
Proteus	transparent colourless colonies
Klebsiella	transparent colourless colonies
Enterococcus	small red colonies
Staphylococcus	small pink colonies

Quality assurance (37°C - 24hrs)

Productivity Control
E.coli ATCC 25922: growth, red pink colonies
E.faecalis ATCC 29212: growth, small red colonies

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

References
Dept. of Health Social Security (1969) - The bacteriological examination of water supplies. 4th Ed. MHSO, London.
Windle Taylor, E. (1958) - The examination of waters and water supplies. 7th Ed. Churchill Ud., London.
World Health Organisation (1963) - International Standards for Drinking Water, 2nd Ed. WHO, Geneve.