Cetrimide Agar (U.S.P.)

Used for the isolation of Pseudomonas aeruginosa from pharmacological preparations.

Code: KM1202

Typical formula	**(g/l)**
Gelatin Peptone	20.0
Magnesium chloride	1.4
Potassium sulphate	10.0
Cetrimide	0.3
Agar	13.6

pH: 7.2 ± 0.2

Directions
Weigh 45.3 grams of powder, disperse in 1 litre of deionised water. Add 10mls of glycerol, allow to soak for 10 minutes then swirl to mix. Sterilise at 121ºC for 10 minutes.

Description
A medium recommended by the United States Pharmacopoeia for the isolation of Pseudomonas aeruginosa from pharmacological preparations. Subculture is carried out onto the medium after enrichment in Tryptone Soy Broth. Cetrimide inhibits the growth of many microrganisms whilst allowing Ps. aeruginosa to develop typical colonies, which will fluoresce in ultraviolet light and produce green pigment.

Appearance: Opalescent, pale yellow agar.

Q.C. organisms: *Ps. aeruginosa , E. coli* (inhibition)

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

Inoculation: Subculture from enrichment broth, streak out for single colonies.

Incubation: 30-35ºC aerobically for 24-48 hours.

References
United States Pharmacopoeia XXI. 1985.
Brown V. I., Lowbury E. J. L. 1965. Use of an improved Cetrimide Agar Medium and other culture methods for Pseudomonas aeruginosa. J. Clin. Pathol. 18, 752-756.

CHAPMAN STONE MEDIUM

A selective medium for the isolation and presumptive identification of staphylococci

Code: KM3002

Typical formula	**(g/l)**
Tryptone	10.0
Yeast Extract	2.0
Gelatin	30.0
D-Mannitol	10.0
Sodium Chloride	55.0
Ammonium Sulphate	75.0
Dipotassium Phosphate	5.0
Agar	15.0

pH 7.0 +/- 0.2

Directions
Suspend 202g in 1000 ml of cold distilled water: heat to boiling heat with frequent agitation and autoclave at 121°C for 15 minutes.

Description and Method
Chapman Stone Medium is a selective medium, prepared according to the original formula of Chapman, for the isolation and presumptive identification of staphylococci.
Inoculate the medium by streaking the specimen onto the surface and incubate at 37°C for 24-48 hours. Chapman Stone Medium is used for gelatin hydrolysis and mannitol fermentation tests. The selectivity of the medium is due to the high content of NaCl (5,5%) that allows a good growth of *Staphylococcus aureus.* For this reason Chapman Stone Medium is used for the isolation of staphylococci from foods suspected of being the cause of food poisoning. *S. epidermidis* also grows on Chapman Stone Medium even though it has different characteristics (white or non-pigmented colonies, with or without a clear zone) from *S. aureus. Streptococcus faecalis* is partially inhibited while Gram-negative bacteria are completely inhibited. The inoculated medium is opaque and whitish in colour, owing to precipitates that form between the gelatin and ammonium sulphate. The medium appears clear around the colonies of microorganisms that have liquefied the gelatin.
Mannitol is present in the medium as a fermentable carbohydrate. *S. aureus* ferments the mannitol producing acidification of the medium around the colonies. To determine this acidification, typical colonies are removed and a drop of Bromocresol purple is added to the area. To confirm the identification of *S. aureus,* carry out the coagulase test and/or thermonuclease test.

User quality assurance (37°C-48 hrs)

Productivity Control
S.aureus ATCC 25923: growth
S.epidermidis ATCC 12228 growth

Selectivity control
P.mirabilis ATCC 12453: partially inhibited

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

Reference
Chapman (1948) - Food Research; 13 100

CHRISTENSEN AGAR BASE

For the differentiation of a variety of microorganisms on the basis of urease production

Code: KM7277

Typical formula	**(g/l)**
Peptone	1.0
Glucose	1.0
Sodium Chloride	5.0
Monopotassium Phosphate	2.0
Phenol Red	0.012
Agar	15.0

Final pH 6.8 +/- 0.2

Directions
Suspend 24g in 950ml of cold distilled water, heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 45°C and add under aseptically 50ml of Urea 40% Solution. Mix well and dispense the complete medium in quantities of 10ml into sterile tubes or bottles. Allow to cool in a sloping position.

Description
Christensen Agar base is prepared according to the formulation recommended by ISO/DIS 6579. Christensen Agar Base is used to detect the production of urease by *Proteus*, *Klebsiella* and certain yeasts such as *Cyiptococcus* and as an identification test for the differentiation of *Salmonella* spp. (urease negative).

Method
Streak the agar slope surface with a pure culture to be tested. Do not inoculate the butt, to have the control colour of the negative reaction. Incubate at 37°C for 18-24 hours and examine at intervals. If the reaction is positive, splitting of urea liberates ammonia, which changes the colour of phenol red to rose-pink and later to deep cerise. The reaction is often apparent after 2-4 hours.

References
Christensen, W.B. (1946). J. Bact., 52,461466
ISO/DIS 6579 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Salmonella spp. 2000.

CHROMOGENIC CANDIDA ALBICANS AGAR

A chromogenic selective and differential medium for the isolation of *Candida* spp. and the immediate identification of *C. albicans.*

Code: CKM205

Typical formula	**(g/l)**
Growths Factors	18.50
Chloramphenicol	0.05
Gentamicin	0.10
Tryptone	20.00
Glucose	1.00
Agar	13.00
Chromogenic Substrate	0.10

pH: 6.3 +/- 0.2

Directions
Suspend 52.75 in 1000ml of cold distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 115°C for 15 minutes. Cool to 50°C and distribute into sterile petri dishes.

Description
Chromogenic Candida albicans Agar is a selective and differential medium for the isolation of *Candida* spp. and the immediate identification of *C. albicans.* The selectivity of the medium is obtained by the presence of chloramphenicol and gentamicin. The differentiation is obtained by the presence of a chromogenic substrate in the medium to detect a specific enzymatic activity (beta-glucosaminidase) of *C. albicans*.

Directions
Suspend 52.75 in 1000ml of cold distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 115°C for 15 minutes. Cool to 50°C and distribute into sterile petri dishes. Final pH: 6.3 +/- 0.2

Method
The medium can be employed according to usual laboratory techniques. *C. albicans* grows with blue or green-blue colonies after incubation at 37°C for 24 - 48 hours. Other species of the *Candida* genus grow with colourless colonies

Quality Control(25°C-72 hrs)

Productivity control
C.albicans ATCC 18804: growth

Selectivity Control
E.coli ATCC 25922: inhibited

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

CHROMOSENSE SALMONELLA AGAR BASE

A chromogenic medium, + selective supplements for the isolation and identification of *Salmonella* spp. including *S.typhi.*

Code: SKM105

Typical Formula	**(g/l)**
Peptone	10.0
Selective Compounds	12.0
Chromogenic Mixture	0.9
Agar	15.0

Add: Salmonella Selective Supplement Vial A (each vial sufficient for 500 ml of medium) **Code: KMS5125A**

Emulsifying agents ……..5.7ml

Add: Salmonella Selective Supplement Vial B (each vial sufficient for 500 ml of medium) **Code: KMS5125B**

Cefsulodin…… 2.5mg

Final pH 7.2 ± 0.2

Directions
Suspend 19g in 500 ml of cold distilled water; add the contents of one vial of Salmonella Selective Supplement Vial A, heat to boiling and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and add the contents of one vial of Salmonella Selective Supplement Vial B reconstituted with 2ml of sterile distilled water. Mix well and pour into sterile petri dishes.

Description
Chromogenic Salmonella Agar is a selective and diagnostic medium useful for the isolation and identification of *Salmonella* spp. from clinical specimens, foodstuff and environmental samples. The selectivity of the medium is improved by a cephalosporin which inhibits the growth of *Pseudomonas*, by sodium deoxycholate which suppress the growth of Gram-positive and some Gram-negative bacteria and by Tergitol 4 which is active mainly against the growth of *Proteus* spp. The differentiation between the salmonella and non-salmonella colonies is achieved by a chromogenic substrate for a specific esterase enzyme of *Salmonella* that is split with the liberation of an insoluble magenta-red dye. A chromogenic glucopyranoside derivative, which is split by beta-glucosidase due to the formation of an insoluble blue-green dye.
The chromogenic and selective compounds of the medium allow the detection also of the rare lactose positive *Salmonella* strains. Chromogenic Salmonella Agar is useful also for the detection of *Salmonella typhi.*

Method
Chromogenic Salmonella Agar can be used according to the normal laboratory practices for *Salmonella* isolation with direct plating or after the enrichment in non-selective and selective liquid media. Incubate the

inoculated plates at 37°C for 18-24 hours and observe for the presence of typical magenta-red colonies. The growth characteristics of different species are summarised in the following table:

Microorganism	**Growth characteristics**
Salmonella spp.	Good growth, magenta-red colonies
Salmonella spp. lac+	good growth, magenta-red colonies
Salmonella typhi	good growth, magenta-red colonies
E.coli	poor growth with colourless colonies
Enterobacter spp.	growth with blue-green colonies
Klebsiella spp.	poor growth with blue-green colonies
Pseudomonas spp.	inhibited
Proteus spp.	poor growth with pale brown or green colonies
Gram-positive bacteria	inhibited

The sensitivity of *Salmonella* detection with Chromogenic Salmonella Agar is very high, thus all the plates without typical colonies can be rejected with no further identification tests. The plates with typical (magenta-red) growth must be retained and the colonies submitted to biochemical and/or serological confirmation. Sometimes rare strains of *Pseudomonas* and *Aeromonas* can cultivate with magenta-red colonies. These strains can be easy differentiated with a simple oxidase test.

User quality assurance (24 h-37°C)

Productivity control
S.typhimurium ATCC 14028: growth, magenta-red colonies

Specificity control
E.aerogenes ATCC 13048: poor growth, blue colonies

Selectivity control
P.aeruginosa ATCC 27853: inhibited

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

CHROMOGENIC URINARY TRACT AGAR

A chromogenic medium for the isolation, enumeration and direct identification of urinary tract pathogens such as *E.coli*, KES, enterococci, staphylococci.

Code: OKM405

Typical formula	**(g/l)**
Peptone mix	18.7
L-Tryptophan	2.0
Chromogenic mix	13.8
MUG	0.1
Agar	12.0

pH 6.8 +/- 0.2.

Directions

Suspend 46.6g in 1000ml of cold distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and distribute into sterile petri dishes. Use within 48 hours from the preparation.

Description

Chromogenic Urinary tract Agar is a diagnostic medium useful for the isolation, enumeration and direct presumptive rapid identification of urinary tract pathogens: *E.coli, Enterobacter- Klebsiella- Serratia* (KES) *, Proteus-Morganella-Providencia,* Enterococci, Staphylococci. The differentiation between the different bacterial species or genus is achieved by:

1. a chromogenic substrate for beta-galactosidase (GAL) which is split with the liberation of an insoluble pink dye.
2. a chromogenic glucopyranoside derivative which is split by beta-glucosidase (GLU) with the formation of an insoluble blue-green dye.
3. MUG for the fluorogenic detection of beta-glucuronidase enzyme (GUR).
4. L-Tryptophan for the detection of tryptophan deaminase (TDA) of *Proteus* spp. *Morganella* spp., *Providencia* spp. and for indole test of *E.coli* colonies.
5. The opaque white background of the medium enhances the colour of the colonies.

Method

Chromogenic Urinary Tract Agar (CUTA) can be used according to the usual laboratory practices for urine bacterial count, by spreading the specimen on the agar surface and incubating at 37°C for 18-24 hours. The cultivated colonies can be identified with the following scheme:

Colonies on CUTA	**Enzyme activity**	**Additional characterisation on CUTA**	**Enzyme activity**	**Results**
Large pink colonies	GAL +	fluorescent under Wood lamp indole positive	GUR + IND +	*E.coli*
Pink colonies	GAL +	not fluorescent under Wood lamp	GUR -	supplementary identification needed
Small blue-green	GLU +			Enterococci

colonies	GAL -	*St.agalactiae*
Blue to purple	GLU +	*Klebsiella, Enterobacter,*
colonies	GAL +	*Serratia*
Brown colonies	TDA +	*Proteus-Morganella-Providencia*
with brown halo	GLU -	glucosidase negative strains
Blue colonies	TDA +	*Proteus-Providencia*
with brown halo	GLU +	glucosidase positive strains
Colourless		supplementary identification tests
Colonies		needed

Notes:

- It is reported that some strains of the bacterial genus reported above have abnormal biochemical patterns.
- Between the *Proteus-Morganella-Providencia* group, *P.mirabilis* is indole negative and can be easy recognised
- Biochemical identification is needed for species identification within *Klebsiella Enterobacter Serratia* (KES) group
- A Pyroglutamate (PYR) test might be necessary to differentiate enterococci from *S.agalactiae*
- *S.saprophyticus* and *S.xylosus* produces small pink colonies and are MUG negative.
- Biochemical tests are necessary for species identification of micro-organisms producing colourless colonies.
- Gram staining is recommended to confirm any doubtful colour reactions.

User quality assurance (24 h-37°C)

Productivity control
E.coli ATCC 25922: growth, large pink colonies fluorescent under a Wood's lamp
E.aerogenes ATCC 13048: growth, blue colonies not fluorescent under a Wood's lamp
S.aureus ATCC 25923: growth, colourless colonies

Storage
Dehydrated medium: 2-8°C
User prepared plates: 24 hours at 2-8°C

CHROMOGENIC E.COLI O157 AGAR

A selective chromogenic medium for the detection of *E.coli* O157

Code: EKM605

Typical formula (g/l)
Peptone mix.....17.0
Bile salts No.3.....1.5
Agar.....12.0
Chromogenic Mixture.....0.5

pH 7.2 +/- 0.2

Directions
Suspend 31g in 1000 ml of cold distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and distribute into sterile petri dishes.

Description
Chromogenic E.Coli O157 Agar is a selective and differential medium for the isolation and immediate identification of *E.coli* O157. The selectivity of the medium is obtained by the presence of Bile Salts No.3, thus Gram-positive bacteria are completely inhibited. The *Enterobacteriaceae* differentiation is obtained by the presence of a chromogenic mixture, which allows the detection of *E.coli* O157, which develops on the medium with purple colonies. *E.coli* strains not belonging to O157 serotype, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Serratia* grow with blue-green colonies. *E.hermanni* grow with colourless colonies.

Method
Use the medium with the normal laboratory procedures, incubating the inoculated plates at 37°C for 24 +/- 2 hours. Confirm the identification with the appropriate immunological tests.

User quality assurance (24 h-37°C)

Productivity control
E.coli O 157 ATCC 43888: growth, purple colonies

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

Selectivity control
E.faecalis ATCC 19433: inhibited

Storage
Dehydrated medium: 2-8°C
User prepared plates: 24 hours at 2-8°C

CHROMOGENIC COLIFORM AGAR

Chromogenic powdered and ready to use medium for the simultaneous detection of coliforms and *Escherichia.*

Code: KM2992

Typical formula (g/l)
Tryptose…….10.00
Tryptophan……..0.10
Peptone mix..…….5.00
Yeast Extract……..3.00
Sodium Chloride…….…5.00
Bile Salts no.3..……..1.50
IPTG……0.10
X-GLUC…….……...…..0.06
Salmon GAL…….……..…0.15
Agar……...…13.00

pH 7.0 +/- 0.2

Directions
Suspend 37.9 g in 1000 ml of cold distilled water. Boil until complete dissolution. Distribute and autoclave at 115°C for 15 minutes. For flasks; dissolve the contents of the bottle by boiling in a temperature controlled water batch. Cool to 50°C and distribute into sterile petri dishes.

Description
Chromogenic Coliform Agar is a selective and differential medium for the simultaneous detection of *E. coli* and coliform bacteria in waters and foods. The medium is made selective by the presence of bile salts; the differentiation between coliforms and *E. coli* is given by the presence of Salmon-GAL, a chromogenic substrate for the detection of β-galactosidase and X-GLUC, a chromogen substrate for the detection of β- glucuronidase. Salmon-GAL is hydrolysed by coliforms releasing a salmon colour pigment; this reaction is strengthened in the medium by the presence of IPTG (isopropil-β-D-thiogalactopiranoside). X-GLUC is hydrolysed, among enterobacteria, by *E. coli*, and by a few other strains of *Salmonella* and *Shigella* releasing a blue pigment. The presence of tryptophan in the medium allows testing the indole directly onto the colonies by adding Kovac's Reagent, for the confirmation of *E. coli*.

Method
Carry out the simultaneous detection of coliform bacteria and *E. coli*, following the usual methods with surface streaking, poured plate or MF techniques and with incubation at 37°C for 18-24 hours: The colonies appearance is the following: *Escherichia coli*: dark blue colonies, indole positive Coliforms (other than *E. coli*); salmon coloured colonies. *Proteus*: colonies with bright brown halo. Other *Enterobacteriaceae*: colourless colonies. Gram-positive bacteria are usually inhibited. The Indole test is carried out by adding about 1ml of Kovac's Reagent to the colonies and observing the formation of a red colour within 1-2 minutes.

User quality assurance (24 h-37°C)

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

Specificity control
E.aerogens ATCC 13048: growth, salmon colonies

Selectivity control
E.faecalis ATCC 19433: inhibited

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

CEC AGAR

A chromogenic and fluorogenic medium for the simultaneous detection of total coliforms and *Escherichia coli* in water and foodstuffs.

Code: PKM805

Typical formula	**(g/l)**
Tryptose	10.00
Tryptophan	1.00
Peptone mix	5.00
Yeast Extract	3.00
Sodium Chloride	5.00
Bile Salts no.3	1.50
IPTG	0.10
X-GAL	0.08
MUG	0.05
Agar	13.00

pH 7.4 ± 0.1

Directions
Suspend 38.8g in 1000ml of cold distilled water. Heat to boiling, stirring until complete dissolution. Sterilise in the autoclave at 115°C for 15 minutes. Pour into sterile petri dishes and use within one week.

Description
The detection and enumeration of faecal indicators is one of the main tests for estimating the microbiological quality of waters and foodstuffs. The usual methods require from 24 till 72 hours to get complete results, and give some false positive and false negative or doubtful results due to late or weak lactose metabolism. CEC Agar allows a quantitative detection in 18-24 hours of total coliforms and *E.coli* through a method based on the enzymatic hydrolysis of fluorogenic and chromogenic substrates by means of ß-galactosidase (ß-GAL) and ß-glucuronidase (ß-GLU).CEC Agar is prepared with a selective agar base, supplemented with 5-Bromo-4 chloro-3-indolyl-ß-D- galactopyranoside (X-GAL), 4-methylumbelliferil-ß-D-glucuronide (MUG), isopropyl-ß-D-thiogalactoside (IPTG) and Tryptophan. This medium allows the selective growth of *Enterobacteriaceae* and of a few other Gram-negative bacteria, being Gram positive inhibited by the selective agents present in the substrate.
Among *Enterobacteriaceae*, the coliforms have the beta-galactosidase enzyme, hydrolyse the X-GAL compound, and grow with blue green colonies; *E. coli* hydrolyses X-GAL and MUG with formation of umbelliferone, strongly fluorescent when the plates are observed under Wood's lamp. The hydrolysis of X-GAL in enhanced by IPTG. The indole test can confirm the presence of *E. coli* by adding a drop of Kovacs' reagent to the colonies.

Method
CEC Agar can be used with plating method for foodstuffs and with membrane filtration method for water samples. It is useful for the detection and enumeration of:
- *E. coli* and total coliforms in water and foodstuffs incubating at 35 or 37°C for 18-24 hours
- *E. coli* and faecal coliforms in water and foodstuffs incubating at 44°C for 18-24 hours

E. coli grows on the medium with blue-green colonies, fluorescent when observed under a Wood's lamp, positive to indole test.
Coliform bacteria other than *E.coli* grow with blue-green colonies not fluorescent under Wood's lamp.

User quality assurance (24hrs -37°C)

Productivity control
E.coli ATCC 25922: growth, blue-green colonies fluorescent under Wood's lamp

Specificity control
K.pneumoniae ATCC 27736: growth, blue-green colonies not fluorescent under Wood's lamp

Selectivity control
E.faecalis ATCC 19433: inhibited

Storage
Dehydrated medium: 2-8°C
User prepared plates: 1 month at 2-8°C
User prepared flasks: 3 months at 2-8°C

References
Damare, J.M., Campbell, D.F., Johnson, R. (1985) J. Food Sciences 50, 1736
Jermini, M., Domeniconi, F., Jaeggll, M., 1994. C-EC-Agar, a Modified mFC-Agar for the Simoultaneous Enumeration of Fecal Coliforms and E. coli in water samples. Letters in App. Microbiol. 19, 332-335

CHROMOGENIC ECX - GLUC AGAR (*E. COLI* AGAR)

A chromogenic medium for the detection of *E. coli* in water and foodstuff

Code: EKM605

Typical formula	**(g/l)**
Tryptone	20.00
Yeast Extract	5.00
Bile Salts No. 3	1.50
Disodium Phosphate	5.00
Monopotassium Phosphate	1.50
Sodium Chloride	5.00
X-GLUC	0.06
Tryptophan	1.00
Agar	12.00

pH 7.0 +/- 0.2

Directions
Suspend 51g in 1000ml of cold distilled water. Heat to boiling, with agitation until complete dissolution and autoclave at 115°C for 15 minutes. Cool to 50°C and distribute into sterile petri dishes.

Description
ECX-GLUC Agar (Chromogenic E. coli), is a selective differential medium for the enumeration and immediate identification of *Escherichia coli* mainly in water samples by means of MF technique. It is also useful for the detection of *E.coli* in foodstuffs with surface inoculated plate or with poured plate technique. The medium contains bile salts for the complete inhibition of Gram-positive bacteria and X-GLUC (5-bromo-4-chloro-3-indolyl β -D-glucuronide) for the detection of β –glucuronidase enzyme. Among the *Enterobacteriaceae* only *E. coli*, together with some strains of *Salmonella* and *Shigella*, is a β -glucuronidase positive species, so cultivates on the plates with blue or green-blue colonies. β-glucuronidase negative bacteria grow with colourless colonies. It is also possible to carry out the rapid indole test by leaving a drop of Kovacs' reagent onto the medium and observing the reagent turning to red.

Method
Membrane filtration method :

1. Filter the sample on a 0,45µ membrane and settle the last over the medium surface.
2. Tightly close the plate and Incubate at 44 (+/- 0,5)°C for 24 (+/-2) hours.
3. Count as *E. coli* all the blue or blue-green colonies, confirmed by indole test.

Poured plate method:

1. Pour 1ml of the decimal dilutions of the sample into the plates.
2. Add about 15ml of ECX-GLUC Agar pre-cooled to 48-50°C.
3. Mix well the inoculum with the medium.
4. Incubate at 44 (+/- 0,5)°C for 24 (+/-2) hours
5. Count as *E. coli* all blue or blue-green colonies, confirmed by indole test
6. Report the results as UFC/g considering the "dilution factor"

The medium can be inoculated even in surface, with the usual methods.

User quality assurance (44°C - 24hrs)

Productivity control
E.coli ATCC 25922: growth, blue-green colonies indole positive

Specificity control
S.typhimurium ATCC 14028: growth, colourless colonies indole negative

Selectivity control
E.faecalis ATCC 19433: inhibited

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

References
Delisle, G.J., Ley, A. (1989) J. Clin. Microbiol. 27, 778
Frampton, E.W., Restaino, L., Blazko, N. (1988) J. Food Proct. 51,402

E.C.O.A. AGAR

A Chromogenic medium for the detection of enterococci

Code: EC4301

TYPICAL FORMULA (g/l)
Peptones……..26.0
Sodium chloride…….8.5
Growth Factors..... 7.3
Phosphate buffer…….5.0
Agar..... 13.0
Chromogenic Substrates.....200.0 mg
Selective compounds…… 27.0 mg

Kanamycin Selective Supplement - (FOR 500 ML OF MEDIUM):
Kanamycin Sulphate……..10 mg

pH 7.2 +/- 0.2

DIRECTIONS
Suspend 30g in 500ml of cold distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and add the contents of one vial of Kanamycin Selective Supplement reconstituted with 5 ml of sterile distilled water. Mix well to suspend the flocculent blue precipitate and distribute into sterile Petri dishes.

DESCRIPTION
E.C.O.A. AGAR is a selective and differential medium, for the isolation, enumeration and immediate identification of enterococci, from foodstuffs, environmental samples and clinical specimens. The selectivity of the medium is obtained by the presence of a mixture of antibiotic compounds including kanamycin. The differentiation of the colonies is obtained by the presence of a chromogenic mixture to detect specific enzymatic activities of enterococci and of contaminating bacteria. The medium can be used according to usual laboratory techniques with surface inoculation technique, pouring plated technique or with MF procedure for liquid samples. Enterococci grow with blue or green-blue colonies after incubation at 37°C for 24 hours. The rare non-enterococci strains resistant to the selective agents grow with violet colonies.

User quality assurance (37°C-24 hr)

Productivity control
E.faecalis ATCC 19433: good growth, green-blue colonies

Selectivity control
E.coli ATCC 25922: inhibited

Storage
Dehydrated medium: 15-30°C
User prepared plates and flasks: one week at 2-8°C

Chromogenic Tryptone Bile X-Gluc (TBX) Agar

A chromogenic medium for the detection of *E. coli* in foodstuff

Code: KM1115XG

Typical formula	**(g/l)**
Tryptone	20.0
Bile Salts No. 3	1.5
Agar	14.0
X-GLUC	75.0 mg

pH 7.2 ± 0.2

Directions
Weigh 35.6g in 1000ml of cold distilled water. Heat to boiling with agitation until complete dissolution and autoclave at 121°C for 15 minutes. Cool to 50°C and distribute into sterile Petri dishes.

Description
Chromogenic Tryptone Bile X-GLUC (TBX) Agar is a selective differential medium for the enumeration and immediate identification of *Escherichia coli* in foodstuff. It is prepared according to the formulation given by ISO 16649-2. The medium contains bile salts for the complete inhibition of Gram-positive bacteria and X-GLUC (5-bromo-4-chloro-3 indolyl â-D-glucuronide) for the detection of â-glucuronidase. Among the *Enterobacteriaceae* only *E. coli*, together with some strains of *Salmonella* and *Shigella*, is one of the few â-glucuronidase positive species, so cultivates on the plates with blue or green-blue colonies. â- glucuronidase negative bacteria grow with colourless colonies.

Method
Transfer 1ml of the test sample into a sterile Petri dish if liquid, or 1ml of the initial suspension (10^{-1}), in the case of other products. Inoculate 2 plates per dilution. Repeat the procedure with further decimal dilutions if necessary. Pour into each Petri dish about 15ml of TBX Agar pre-cooled to 47-50°C. Mix inoculum well with the medium. Invert the inoculated dishes and incubate at 44°C for 18-24 hours. In cases where stressed colonies are suspected incubate for 4 hours at 37°C before incubation at 44°. Do not incubate over 45°C. Count as presumptive all *E. coli* the blue or green-blue colonies. Report the results as UFC/g considering the “dilution factor”. The medium can be inoculated even on the surface with the usual methods.

Quality Assurance (44°C - 24 hrs)

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

Specificity control
S.typhimurium ATCC 14028: growth, colourless colonies

Selectivity control
E.faecalis ATCC 19433: inhibited

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

Reference
ISO 16649-2 (2001). Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *E.coli*- part 2: colony count technique at 44°C using 5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid.

CLED MEDIUM (Single Indicator)

A non-inhibitory medium for the total microbial count in urine tract infections

Code: KM1018

Typical formula	**(g/l)**
Peptone	7.00
Tryptone	4.00
Lactose	10.00
L-Cystine	0.128
Bromothymol Blue	0.020
Agar	15.00

pH 7.3 +/- 0.2

Directions
Suspend 36g in 1000 ml of cold distilled water. Heat to boiling and autoclave at 121°C for 15 minutes.

Description
Cystine Lactose Electrolyte Deficient (CLED) Medium, prepared according to a modification of the original formula of Sandy's, is a non-inhibitory medium particularly recommended for the total microbial count in urine. The medium supports the optimal growth of urinary pathogens, and provides a clear differentiation of the colonies. The lactose is present in the medium as a fermentable carbohydrate; lactose-fermenting bacteria acidify the medium with a colour change of the indicator (Bromothymol Blue) to yellow. CLED Medium is deprived of electrolytes to prevent swarming of *Proteus* spp. The medium is used onto plates, bi and tri-plates or on dip-slides, coupled with MacConkey Agar for rapid evaluation of the number of bacteria in the urine.

Quality assurance (37°C-24hrs)

Productivity control
E.coli ATCC 25922: growth, yellow colonies
S.aureus ATCC 25923: growth, uniform deep yellow colonies
P.vulgaris ATCC 8427:growth, bluish colonies, spreading inhibited

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

References
Mackey J. P. & Sandy's, G.H. (1960's) J.Med. Lab. Techn. 17 224

CLED MEDIUM with Andrade Indicator (BEVIS)

A non-inhibitory medium recommended for the total microbial count in urine tract infections with Bevis modification to formulation.

Code: KM1019

Typical formula	**(g/l)**
Peptone	7.00
Tryptone	4.00
Lactose	10.00
L-Cystine	0.128
Bromothymol Blue	0.020
Andrade indicator	0.100
Agar	15.00

pH 7.5 +/- 0.2

Directions
Suspend 36g in 1000 ml of cold distilled water; heal to boiling to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Cystine Lactose Electrolyte Deficient (CLED) Medium with Andrade indicator is a non-inhibitory medium, particularly recommended for the total microbial enumeration in urine. It differs from CLED medium because of the introduction in the formula of the Andrade's indicator, which allows a better differentiation of the colonies. Lactose is present in the medium as a fermentable carbohydrate. The lactose fermenting bacteria acidify the medium in a more or less intense way, with a colour change of Andrade indicator to different red tones and bromothymol blue to yellow; the lactose non-fermenting bacteria cause an alkalisation of the substrate with a colour change of the indicators to blue-green.

Quality assurance (37°C-24hrs)

Productivity control
E.coli ATCC 25922: good growth, red colonies with red halo
S.aureus ATCC 25923: good growth, light pink, small colonies
P.vulgaris ATCC 8427: good growth, green-blue, not swarming colonies

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

References
Bevis, T. D. A modified electrolyte-deficient culture medium, (1968) J.Med. Lab. Techn. 25: 38-41.

CLOSTRIDIUM AGAR

Used for the detection and enumeration of the spores of clostridia

Code: KM5432

Typical formula	**(g/l)**
Yeast Extract	3.0
Beef Extract	10.0
Tryptone	10.0
Glucose	5.0
Soluble Starch	1.0
Sodium Chloride	5.0
Sodium Acetate	3.0
Cystine HCl	0.5
Agar	15.0

CLOSTRIDIUM BROTH

Used for the detection and enumeration of the spores of clostridia

Code: KM5442

Typical formula	**(g/l)**
Yeast Extract	3.0
Beef Extract	10.0
Tryptone	10.0
Glucose	5.0
Soluble Starch	1.0
Sodium Chloride	5.0
Sodium Acetate	3.0
Cystine HCl	0.5
Agar	0.5

pH 6.8 +/- 0.2

Directions
Suspend 52.5g of Clostridium Agar and 38g of Clostridium Broth in 1000 ml of cold distilled water. Heat to boiling, distribute (in the case of Clostridium Broth distribute 25ml into screw-capped bottles with a capacity of 25 ml), and sterilise by autoclaving at 115°C for 20 minutes. For the examination of water samples, prepare the Clostridium Broth at double strength reducing the water volume by half and transfer 10ml and 50ml aliquots into screw-capped bottles with a capacity respectively of 25 and 100ml. Prepare a 4% solution of sodium sulphite and a 7% solution of ferric citrate; if necessary, heat the ferric citrate solution for 5 minutes to dissolve completely. Sterilise the two solutions by filtration, and store at 2-5 °C in closed bottles; the two solutions are stable for two weeks. The day of analysis, mix equal volumes of the two solutions and, under sterile conditions, add 0.5ml of reagent to each 25ml of medium. For the preparation of double strength Clostridium Broth add 0.4ml of the mixture to each 10ml and 2ml to each 50ml.

Description
Clostridium Broth, which corresponds in the formulation to Differential Reinforced Clostridial Medium reported in ISO 6461/1 and to Reinforced medium for clostridia recommended by EP, is a non-selective medium used for the detection and

enumeration of the spores of sulfite-reducing anaerobes (clostridia) in water and foodstuffs by the MPN method. The medium is very rich and permits the growth of most clostridia, and many other anaerobes and facultative anaerobes. To demonstrate the growth of clostridia, sodium sulphite and iron citrate may be added to the medium. However, blackening of the medium, due to the reduction of sulphite to sulphur, and of its precipitation in the form of iron salts, is not specific for the *Clostridium* genus, because other bacteria, such as *Salmonella, Proteus,* and some strains of *Escherichia coli* give the same reaction. However, because these latter microorganisms are asporigens, pasteurisation and ethyl alcohol treatment of the specimen eliminates them completely.

Method

For the presumptive enumeration of the clostridia in water ISO 6461/1 reports the following technique: Heat the sample in a water batch at 75 +/- 5°C for 15 minutes recording the temperature with a thermometer. Add 50ml of sample to a screw-cap bottle containing 50ml of double strength complete Clostridium Broth. Add 10ml of sample to a series of five screw-cap bottle containing 10ml of double strength complete Clostridium Broth. Add 1ml of sample to a series of five screw-cap bottle containing 25ml of single strength complete Clostridium Broth

If necessary add 1ml of 10^{-1} dilution of the sample to a series of five screw-cap bottle containing 25ml of single strength complete Clostridium Broth. In order to carry out a qualitative examination of 100ml of water without making an MPN count, use a 200ml vial filled with a mixture of 100ml of double strength complete Clostridium Broth and 100ml of sample. If necessary top up all the bottles with a single strength complete Clostridium Broth to bring the volume of liquid level with the neck and to ensure that only a very small volume of air remains, then seal the bottles hermetically, or incubate under anaerobic conditions. Incubate the inoculated bottles at 37°C for 44 hours. Large volume of culture in hermetically sealed glass bottles may explode due to gas production. The addition of iron wire, heated to redness and placed into the medium before inoculation, may aid anaerobic conditions. Bottles, in which a blackening effect occurs as a result of the reduction of sulphite, shall be regarded as positive. Express the results in accordance to ISO 8199.

Clostridium Agar has the same characteristics as Clostridium Broth, and it is recommended for the isolation of clostridia in pure culture, starting from their growth in bottles of Clostridium Broth. For this, Freame and Fitzpatrick recommend the use of open-ended test tubes (120x13 mm) and plugged at one end with a cotton wad and at the other with a rubber plug. 1ml of a blackened culture broth of Clostridium Broth is introduced into these test tubes, which are then filled with Clostridium Agar. They are incubated at 30°C until discrete white (non-clostridial types) or black colonies are visible in the agar. For the isolation of clostridia in pure culture, choose the colonies to be subcultured, open the test tube at the rubber plug end, push the agar column outwards by means of the cotton wad until the desired colony is clear of the test tube. Remove the agar under sterile conditions and transfer the colony into Clostridium Broth with a Pasteur pipette. The culture broth derived from it is tested for purity on a plate under anaerobic conditions or, if necessary, with a new subculture in Clostridium Agar test tubes. Identification of the pure culture can be carried out using the Willis and Hobbs, or the Smith and Holdeman procedure.

Quality assurance (37°C-48 hrs, anaerobic incubation)

Productivity control

C.perfringens ATCC 13124: black growth

C.sporogenes ATCC 19404: black growth

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

References
Freame, B. & Fitzpatrick, B.W.F. (1967) - The use of Differential Reinforced Clostridial Medium for the isolation and enumeration of Clostridial from foods. In “The Society for Applied Microbiology Technical series” No. 5: Isolation of Anaerobes, ed. Shapton, D.A. & Board, R. G. Vol. 5, London: Academic Press., pag. 49-55.
Gibbs, M.B. & Freame B. (1965) - J. Appl. Bact., 28, 95-111.
Eurpean Pharmacopoeia, 3rd ed. 2001 Supplement
ISO 6461/1 (1986) Water quality – Detection and enumeration of the spores of sulfite reducing anaerobes (clostridia) – Part 1: Method by enrichment in a liquid medium
Smith, L.D.S. & Holdeman, L.V. (1968) - The Pathogenic Anaerobic Bacteria. Springfield: Charles C. Thomas
Willis A.T. Hobbs, G. (1959) J. Path. Bact. 77, 511

CLOSTRIDIUM DIFFICILE AGAR BASE

Code: KM1024

A medium base used for the isolation of *Clostridium difficile.*

Typical formula	**(g/l)**
Peptone mix	40.0
Disodium Phosphate	5.0
Monopofassium Phosphate	1.0
Magnesium Sulphate	0.1
Sodium Chloride	2.0
Fructose	6.0
Agar	15.0

Supplement with (vial contents for 500ml of medium)

D-Cycloserine	250 mg
Cefoxitin	8 mg
Defribinated Horse Blood	50 ml

pH 7.4 +/- 0.1

Directions
Suspend 34.5g in 500 ml of cold distilled water; heat to boiling and autoclave at 121ºC for is minutes. Reconstitute one vial of Clostridium Difficile Supplement with 5ml of sterile distilled water and add it to the pre-cooled base. Mix thoroughly and add 25ml of defibrinated horse blood, mix thoroughly and distribute into sterile Petri dishes.

Description
Clostridium difficile was first isolated from meconium and infant faeces in 1935 by Hall and O'Toole but only recently has been seen a clear correlation between the cytotoxin produced by *Clostridium difficile* and the illness characterised by pseudomembranous colitis with inflammatory plaques, serious diarrhoea, dehydration, fever and leucocytosis. The most suitable medium for the selective isolation of *Clostridium difficile* is that proposed by George et al. in 197B. The medium is a slightly modified version of the original formula. It contains fructose, cycloserine, cefoxitin and defibrinated horse blood as a substitute for the egg yolk emulsion, used by George et al. Cycloserine and cefoxitin inhibit enterobacteria, faecal streptococci, anaerobic non spore-forming Gram-negative bacilli and *Clostridium* spp. except for *Clostridium difficile.*

Method
Streak the specimen directly onto the agar surface, to obtain well isolated colonies. Incubate at 37°C for 48 hours in anaerobic conditions. After 48 hours *Clostridium difficile* colonies are visible with the following characteristics: circular, raised, opaque grey, sometimes with irregular borders, 4-6 mm in diameter.

User quality assurance (37°C-48 hrs, anaerobic incubation)

Productivity control
C.difficile ATCC 9689: growth

Selectivity control
E.coli ATCC 25922: inhibited

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

References
Bartlett, J.G. et al. (1978) N. Eng. J. Med., 298, 531.
Hall and E. O'Toole (1935) Am. J. Dis. Child. 49, 390.
Larson, H.E. et al. (1978) Lancet, i. 1063-6

COLUMBIA AGAR BASE

A general purpose nutritious agar base for the preparation of enriched media when sterile blood is added.

Code: KM1021

Typical formula	**(g/l)**
Peptone mix	10.0
Tryptose	10.0
Peptone	3.0
Maize Starch	1.0
Sodium Chloride	5.0
Agar	13.0

pH: 7.3 +/- 0.2

Directions
Suspend 42 g in 1000ml of cold distilled water; heat to boiling and sterilise in the autoclave at 121°C for 15 minutes. Cool to 50 °C and add the suitable enrichment supplement.

Description
Elmer et al. of the Columbia University, found that the combination of meat and casein peptones used, gave better results than those obtained with current blood agar bases: it affords a more rapid and abundant growth of streptococci, staphylococci, *Neisseria* and *Haemophilus,* with better defined haemolytic reactions. Columbia Agar Base can be used as it is, for the cultivation of bacteria, which are not particularly fastidious, or else enriched in various ways:

1. Blood agar: add 5 % sterile defibrinated sheep or horse blood to the base which has been sterilised and cooled in a water bath to 50°C; mix thoroughly and pour into sterile plates.
2. Chocolate agar: add 10% sterile defibrinated sheep or horse blood to the base which has been sterilised and cooled in a water bath to 50°C, heat to 80°C for 10 minutes with constant stirring, cool to 50°C and pour into sterile plates.
3. Serum agar: add 20% serum to the base, which has been sterilised and cooled in a water bath to 50°C. Mix and pour into sterile plates. The medium, thus prepared serves as a virulence test for *Corynebacterium diphtheriae,* using the Elek diffusion technique.
4. Lactose egg yolk milk agar: suspend 41 grams of Columbia Agar Base in 1000 ml of distilled water. Add 12g of lactose, 1g of agar and 3.25ml of a 1% solution of Neutral Red; heat to boiling stirring constantly. Adjust pH to 7.0 and sterilise by autoclaving at 121°C for 15 minutes. Cool in a temperature-regulated water bath to 50°C, and aseptically add 150ml of sterile skim milk and 36ml of sterile egg yolk emulsion. Mix thoroughly and pour into sterile plates. The prepared medium consequently allows for the identification of clostridia on the basis of lactose utilisation, lecithinase and lipase production and proteolytic activity. The addition of 180µg/ml of neomycin sulphate and 240µg/ml of sodium azide make the medium selective for clostridia.
5. Campylobacter Media: add to 500ml of the pre-cooled base, 10% defibrinated or lysed horse or sheep blood and the content of one vial of Baser Wang Antimicrobic Supplement or other suitable selective supplements.

Quality assurance (Columbia Blood Agar Base + 5% def.blood) (37°C-24 h)

Productivity control
S.pyogenes ATCC 19615: good growth, β-haemolysis
S.pneumoniae ATCC 6303: good growth, α-haemolysis
S.aureus ATCC 25923: good growth
E.coli ATCC 25922: growth

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

Reference
Ellner, P.D. Stoessel, C.J. Drakeford, E. & Vasi, F. (1966. Am. J. Clin. Path. 45, 502-504.

COLUMBIA CNA AGAR BASE

A medium for the isolation of Gram-positive cocci

Code: KM3612

Typical formula	**(g/l)**
Peptocomplex	10.0
Tryptose	10.0
Peptone	3.0
Mais Starch	1.0
Sodium chloride	5.0
Agar	2.0
Nalidixic Acid	15 mg
Colistin	10 mg

pH: 7.3 +/- 0.2

Directions
Suspend 41 g in 1000 ml of cold distilled water. Heat to boiling and sterilise in the autoclave at 121°C for 15 minutes. Cool to 50°C and ad 5% of defibrinated blood.

Description
Columbia CNA Agar Base is prepared with the same formula as Columbia Agar Base, with the addition of 10mg/litre of Colistin and 15mg/litre of Nalidixic Acid to inhibit the growth of Gram-negative bacteria. The medium is used with the addition of 5% defibrinated blood to isolate Gram-positive cocci and to demonstrate their haemolytic properties.

Quality assurance (Columbia CNA Blood Agar Base + 5% def.blood) (37°C-24 h)

Productivity control
S.pyogenes ATCC 19615: good growth, β haemolysis
S.pneumoniae ATCC 6305: good growth, α haemolysis
S.aureus ATCC 25923: good growth

Selectivity control
P.mirabilis ATCC 12453: partially inhibited

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

Reference
Quality Assurance for Commercially prepared Microbiological Culture Media-2nd ed.; Approved Standard. 1996.

Cooked Meat Granules

Dried minced ox heart for the preparation of Cooked Meat Medium.

Code: KM1022

Directions:
Put a full scoop of granules into a universal container and add 20ml Nutrient Broth No.2 or Anaerobe Liquid Medium. Sterilise by autoclave at 121ºC for 15 minutes in capped tubes, which should be tightened after autoclaving to prevent re-oxygenation. Medium prepared with Nutrient Broth No.2 should be re-steamed when used after a period of storage. Medium made with Anaerobe Liquid Medium will not require re-steaming after storage.

Storage: up to 6 months at 15-20ºC in the dark.

References: Cruickshank, R. 1972. Medical Microbiology.

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Cooked Meat Medium

A Robertson Cooked Meat Broth for the isolation and cultivation of anaerobic and aerobic organisms.

Code: KM1023

Typical formula	**(g/l)**
Cooked meat	35.0
Beef Extract	10.0
Peptones	10.0
Sodium chloride	5.0

pH: 7.3 ± 0.2

Directions:
Use the calibrated scoop to distribute 1.2g amounts of granules into 25ml tubes or bottles. Add 20ml deionised water. Sterilise at 121ºC for 10 minutes. Use proportionately more granules and water if greater depths of medium are required. Allow to cool before use.

Appearance: Granules covered in slightly opalescent pale yellow liquid.

Inoculation: Samples or swabs directly into the medium.

Incubation: 37ºC for mesophiles, appropriate temperature for thermophiles.

Interpretation: Reddening of meat - saccharolytic organism - Blackening and digestion proteolytic organism.

References
Robertson, M., (1916) J. Path. & Bact. 20 327-348.

CORN MEAL AGAR

A mycological medium that is a suitable substrate for chlamydospore production by *Candida albicans*.

Code: KM5405

Typical formula	**(g/l)**
Corn Meal Extract	2.0
Agar	15.0

PH: 6.0 +/- 0.2

Directions

Weigh 17g in 1000ml of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description

Corn Meal Agar is a mycological medium, which is a suitable substrate for chlamydospore production by *Candida albicans.* The addition of Tween 80® to Corn Meal Agar greatly enhances the development of chlamydospores on the medium.

Method

A single Petri dish containing Corn Meal Agar may be used to identify four or five different colonies of *Candida* grown on Sabouraud Dextrose Agar (SDA). Using a straight wire, pick a colony off the surface of the SDA and make a deep cut in the Corn Meal Agar. Repeat for each colony. Place a flamed sterile cover slip over the line of inoculum. After incubation for 24 to 48 hours at 22°C, the streaks are studied microscopically. Along such streaks, *C. albicans* produces mycelium-bearing ball-like clusters of growing cells and the characteristic thickening around chlamydospores. The addition of 0.001g % w/v Trypan blue to Corn Meal Agar provides a contrasting background for the study of characteristic morphological features of yeast cultures.

Storage

Store the dehydrated medium at 10-30°C and use before the expiry date on the label. Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Pale-white coloured powder.
Prepared medium: Light straw coloured gel.

Quality Control

Positive controls:
Candida albicans ATCC10231

Negative control:
Candida krusei ATCC6258

CZAPEK DOX AGAR (Modified)

Used for the cultivation of saprophytic fungi

Code: KM6455M

Typical formula	**(g/l)**
Sucrose	30.00
Sodium Nitrate	2.00
Potassium Sulphate	0.35
Magnesium Sulphate	0.50
Potassium Chloride	0.50
Ferrous Sulphate	0.01
Agar	12.00

pH 6.8 +/- 0.2

CZAPEK DOX BROTH

Used for the cultivation of saprophytic fungi

Code: KM6456

Typical formula	**(g/l)**
Sucrose	30.00
Sodium Nitrate	2.00
Potassium Chloride	0.50
Mg Glycerophosphate	0.50
Ferrous Sulphate	0.01
Potassium Sulphate	0.35

pH 7.0 +/- 0.2

Directions
Suspend 45.4g of agar in 1000ml of cold distilled water. Heat to boiling, distribute and autoclave at 115°C for 15 minutes, or 35.4g of broth in 1000ml of cold distilled water. Heat to boiling, distribute and autoclave at 121°C for 15 minutes.

Description
Czapek Dox Agar and Broth are semi-synthetic media of defined chemical composition used for the cultivation of saprophytic fungi and bacteria able to use inorganic nitrogen.

The sole source of inorganic nitrogen in the two media is the sodium nitrate, and the sole carbon source is the sucrose. Czapek Dox Broth is especially suitable for the enrichment, cultivation and identification of bacteria and fungi present in soil.

User quality assurance (25°C-72 h)

Productivity control
C.albicans ATCC 18804: growth
A.niger ATCC 9642: growth

Storage
Dehydrated media: 15-30°C

References
Booth, C. (1971) - Methods in Microbiology Vol. 4, London: Academic Press.
Dox, (1910) - U.S. Dept. Ag. Anim. Ind. Bull.: 120, 70.

C.E.M.O. Agar Base (Contagious Equine Metritis Organism**)**

A selective isolation medium for Taylorella equigenitalis the causative organism of contagious equine metritis.

Code: KM2204

Typical formula	**(g/l)**
Tryptone	15.0
Soy Peptone	5.0
Sodium chloride	5.0
Agar	12.0
L-Cystine	0.3
Sodium sulphite	0.2

pH: 7.3 ± 0.2

Directions
Suspend 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. Allow to cool to 80ºC, add 50ml of sterile horse blood and allow to "chocolate". Further cool to 47ºC before adding antibiotic selective agents. Mix well and pour into petri dishes.

The medium is a sugar free base with a mixture of casein and soy peptones as nutrients and with L-cystine and sodium sulphite as supplements and reducing agents. The medium is made selective with the addition of amphoteracin (5 mg/l) and trimethoprim (10 mg/l). Streptomycin (200 mg/l) can also be used but sensitive variants of T. equigenitalis have been described.

Q.C. organisms:
T. equigenitalis
E. coli (inhibition)

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

Inoculation: Surface, streaking out for single colonies.

Incubation: 37ºC in 10% CO_2 for 2-3 days.

References
Atherton, J. G. 1978. Inhibition of the C.E.M. organism in mixed cultures. Vet. Rec. 432.

CAMPYLOBACTER BLOOD FREE MEDIUM BASE (KARMALI)

Code: KM2832

Formulation	**(g/ltr)**
Peptone mix	10.0
Tryptose	10.0
Peptone	3.00
Maize Starch	1.00
Sodium Chloride	5.00
Charcoal	4.00
Hematine	0.032
Sodium Pyruvate	0.10
Agar	14.0
Tris	1.0

pH 7.4 +/- 0.2

CAMPYLOBACTER BLOOD FREE MEDIUM BASE

Code: KM1017

Formulation	**(g/ltr)**
Beef Extract	10.00
Peptone	10.00
Tryptone	3.00
Sodium Chloride	5.00
Charcoal	4.00
Sodium Desoxycholate	4.00
Ferrous Sulphate	1.00
Sodium Pyruvate	0.25
Agar	15.00

pH 7.4 +/- 0.2

Methods

CBFMB PLATING MEDIUM*;* suspend 45g of Campylobacter Blood Free Medium Base in 1000ml of distilled water; heat to boiling with agitation and autoclave at 121°C for 15 minutes. Cool to 50°C and add the contents of one vial of Cefoperazone Supplement. Distribute into sterile petri dishes with frequent stirring.

KARMALI MEDIUM: suspend 24.1 g of Campylobacter Blood Free Medium Base Karmali in 500 ml of distilled water; heat to boiling with agitation and autoclave at 115°C for 15 minutes. Cool to 50°C and add the contents of one vial of Karmali Supplement. Distribute into sterile petri dishes with frequent stirring.

Quality assurance (48 h-42°C, reduced O_2)

Productivity control

C.jejuni ATCC 33291*: growth

Selectivity control
E.coli ATCC 25922*: partially or completely inhibited
*NCCLS M22-A2 recommended strains

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

References
Bolton, F.J., Hutchinson, D.N., Coates, D. (1986) J. App. Bacteriol. 56, 151-157
Bolton, F.J., Robertson, L. (1982) J. Clin. Pathol. 35, 462-467
Karmali, M.A., Simor, A.E., Roscoe, M., Fleming, P.C., Smith, S.S., Lane, J. (1986) J. Clin. Microbiol. 21, 456-459
NCCLS document M22-A2, 1996. Quality Assurance for Commercially prepared Microbiological Culture Media-Second Ed.; Approved Standard

Campylobacter Enrichment Broth

A selective enrichment broth for the isolation of Campylobacter spp. from food, environmental samples and faeces

Code: KM1203

Typical formula	**(g/l)**
Meat Peptone	10.0
Lactalbumin Hydrolsates	5.0
Yeast Extract	5.0
Sodium chloride	5.0
Haemin	10.0mg
Sodium pyruvate	0.5
ketoglutaric acid	1.0
Sodium metabisulphite	0.5
Sodium carbonate	0.6

pH: 7.4 ± 0.2

Directions
Suspend 27.6g of powder, disperse in 1 litre of deionised water and allow to stand for 10 minutes. Swirl to mix and autoclave at 121ºC for 15 minutes. Cool to 47ºC, add 2 vials of C.T.V.C. selective supplement (reconstituted with 5mls of 50% alcohol) and 50 mls of saponin lysed horse blood, mix well and dispense into sterile containers.

Description
The use of a selective enrichment broth enhances the recovery of sub-lethally damaged organisms due to processing of foods, or if small numbers of campylobacters are present in heavily contaminated specimens.
This broth has been shown to give appreciably better results than Preston Broth.

Q.C. organisms:
Campylobacter *jejuni*
E. *coli* (inhibition)

Storage: Capped containers: 7 days at 2-8ºC in the dark.

Inoculation: Food homogenate is added to broth in a ratio of 1:4 (w/v) in screw cap containers leaving a head space of 1.5 cm. For faeces 1 ml of a 10% suspension in Buffered Peptone Water is added to 5 ml of broth.

Incubation: Aerobically at 37ºC for 2-4 hours, followed by a further 14-48 hours at 42ºC.

Subculture: On Campylobacter Blood Free Selective Medium.

Cary Blair Transport Medium

Code: KM5519

Description

A transport medium for the collection and shipment of clinical specimens based on the formulation of Cary and Blair.

FORMULATION	**Grams/ltr**
Disodium hyd. phos.	1.1
Sodium thioglycholate	1.5
Sodium chloride	5.0
Calcium chloride	0.09
Agar-Agar	5.6

pH 8.0 ± 0.2

Directions
Weigh 12.5 grams of powder and add to 1 litre of deionised water (conductivity <10ms). Allow to soak for 10 minutes. Swirl to mix then gently bring to the boil to dissolve the agar. Distribute into small, screw cap bottles and sterilise by immersing in free-steam for 15 minutes. Allow to cool and tighten the screw caps to prevent water loss.