UREASE INDOLE TEST BROTH

Code: KM12500

A medium for demonstrating urease activity and indole production.

Directions:
Suspend 37 g in one litre of distilled water and heat gently to dissolve the medium completely. Distribute into final containers and sterilise at 100 °C for 15 minutes.

Typical formula	**(g/l)**
Peptones	10.00
Sodium chloride	2.30
Urea	20.00
Buffers	4.60
Phenol red	0.012

PH: 6.7 +/- 0.2

Warning**:** The medium is heat sensitive. No further sterilisation is necessary or desirable.

Storage: Store the dehydrated medium below 25 °C and use before the expiry date on the label. Store the prepared medium at 2-8 °C.

Colour:	Pinkish
Appearance:	Homogeneous hygroscopic powder

Quality Assurance:

Positive control
Proteus mirabilis, Escherichia coli

Negative control
Shigella sonnei

References:
Roland at al (1947) Ann. Inst. Pasteur 73: 914.

UVM LISTERIA BROTH BASE

A complete UVM base media for the enrichment and detection of *Listeria monocytogenes* in meat and poultry.

Code: KM6972

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

UVM1 Supplements (vial contents for 500ml of medium)

Acriflavin	6 mg
Nalidixic Acid	10 mg

UVM2 Supplements (vial contents for 500ml of medium)

Acriflavin	12.5 mg
Nalidixic Acid	10.0 mg

Final pH 7.2 +/- 0.2.

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

WARNING**:** UVM1 and UVM2 Supplements contain acriflavin, a possible mutagen. Do not inhale. In case of eye or skin contact wash affected area thoroughly with soap and water.

Description
UVM Liquid media are prepared according to the formulations described by Donnelly and Baigent and by MacCalin and Lee. Media supplemented with UVM1 and UVM2 are used for the “two steps” enrichment of *Listeria* spp. in meat products.

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

Incubate the Selective Agar plates at 37°C for 24-48 hours, examine for typical colonies and carry on with identification tests by means of standard biochemical method.

Note: Techniques for the detection of *Listeria* in foods vary, depending on the material under examination and local laws. Refer to various compendia or to national regulations for the complete procedures.

Quality assurance (37°C-24 h)

Productivity control
L.monocytogenes ATCC 19117: growth

Selectivity control
S.aureus ATCC 25923: inhibited

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

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

UREA AGAR BASE

A medium for the differentiation of a variety of microorganisms on the basis of urease production

Code: KM1121

Typical formula	**(g/l)**
Peptone	1.00
Glucose	1.00
Sodium Chloride	5.00
Monopotassium Phosphate	2.00
Phenol Red	0.012
Agar	15.00

pH 6.8 +/- 0.1

Directions
Suspend 2.4g in 95ml of cold distilled water, heat to boiling with frequent agitation, sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 45°C and add, under aseptic conditions, 5ml of Urea 40% Solution. Mix well and dispense the complete medium in quantities of 10ml into sterile tubes. Allow to cool in a sloping position.

Description
Urea Agar base is prepared according to the formulation recommended by ISO/DIS 6579. Urea Agar Base is used to detect the production of urease by *Proteus*, *Klebsiella* and certain yeasts, such as *Cryptococcus* 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.

Quality assurance (37°C-24 hrs)

Urea positive control
P.mirabilis ATCC 25933

Urea negative control
E.coli ATCC 25922

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

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.

UREA BROTH BASE

A liquid medium for the differentiation of a variety of microorganisms on the basis of urease production

Code: KM1122

Typical formula	**(g/l)**
Yeast Extract	0.10
Monopotassium Phosphate	9.10
Disodium Phosphate	9.50
Phenol Red	0.01

pH 6.8 +/- 0.1

Directions
Suspend 0.9g in 95ml of cold distilled water. Heat to dissolve and sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 45°C and add, under aseptic conditions 5ml of Urea 40% Solution. Mix well and dispense the complete medium in quantities of 3-5ml into sterile tubes.

Description
Urea Broth Base, supplemented with urea solution, is used to differentiate P*roteus* from other enteric bacteria. The medium is prepared in accordance with the formula of Stuart et al. The composition of the medium supports excellent growth of *Proteus,* which, through their urease activity, hydrolyses urea with the production of ammonium ions making the pH of the medium alkaline. The medium having a faint red colour, becomes purple-red when the pH reaches or exceeds the value of 8.1. Other microorganisms also possess urease activity, but do not hydrolyse urea as rapidly and intensely as *Proteus.*

Method
Inoculate a tube of Urea Broth with a single colony of lactose non-fermenting organism and incubate at 35-37°C for 2-6 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.

Quality assurance (37°C-24 hrs)

Urea positive control
P.mirabilis ATCC 25933

Urea negative control
E.coli ATCC 25922

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

References
Cowan & Steel's Manual for the Identification of Medical Bacteria. 2nd edition, revised by S.T. Cowan. Cambridge: University Press. (1974).
Edwards, P.R. & Ewing, W.H. (1972) - Identification of Enterobacteriaceae. 3rd edition. Minneapolis: Burgess Publishing Company.
Stuart, C.A., Von Stratum, E. & Rustigian, R. (1945) - J. Bact., 48, 437.