**DEXTROSE AGAR**

A general purposes medium

**Code: KM5852**

| **Typical formula** | **(g/l)** |
|---|---|
| Beef Extract | 3.0 |
| Tryptose | 10.0 |
| Glucose | 10.0 |
| Sodium Chloride | 5.0 |
| Agar | 15.0 |

pH 7.2 +/- 0.2

**DEXTROSE BROTH**

A general purposes liquid medium

**Code: KM5862**

| **Typical formula** | **(g/l)** |
|---|---|
| Beef Extract | 3.0 |
| Tryptose | 10.0 |
| Glucose | 10.0 |
| Sodium Chloride | 5.0 |

pH 7.2 +/- 0.2

**Directions**
Suspend 43g of Dextrose Agar or 23g of Dextrose Broth in 1000ml of cold distilled water, heat to boiling, distribute and autoclave at 121 °C for 15 minutes.

**Description**
Dextrose Agar and Broth are general purposes media, used for the cultivation of a large variety of microorganisms, including fastidious types, such as streptococci, meningococci and pneumococci. The glucose incorporated in the medium, being rapidly metabolised by many microorganisms, supports good microbial growth. Dextrose Agar can be used as it is, with the addition of 5% of sterile defibrinated blood for the preparation of blood agar, or heated to 80°C for the preparation of chocolate *agar.* The medium is not suitable for demonstrating the haemolytic properties of microorganisms, due to the high glucose content. With the addition of 0.1% agar, Dextrose Broth supports a more rapid and luxuriant growth of pathogenic cocci (pneumococci, meningococci, etc.).

**Quality assurance** (37°C-24 h)

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

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

**BACTERIOLOGICAL D-GLUCOSE**
**(Dextrose)**

**Code: CH1013**

A bacteriological grade anhydrous D-glucose for use in microbiological culture media as a fermentable carbohydrate.

**TYPICAL ANALYSIS**

Loss on drying ..... < 5 %
Solubility (2% solution)…….……. soluble
pH ..... 5.1 – 5.5
Microbiological performance in SDA... passes test

**DNASE AGAR**

A medium for the identification of potentially pathogenic staphylococci by demonstration of DN'ase production.

**Code: KM1028**

| **Typical formula** | **(g/l)** |
|---|---|
| Tryptone | 20.0 |
| Deoxyribonucleic acid (DNA) | 2.0 |
| Sodium chloride | 5.0 |
| Bacteriological Agar | 15.0 |

pH: 7.3 ± 0.2

**Directions**
Weigh 42 grams of powder, disperse in 1 litre of deionised water. Allow to stand for 10 minutes, swirl to mix then sterilise by autoclaving at 121°C for 15 minutes. Allow to cool to 47°C then pour into petri dishes.

**Description**
DNase agar provides a convenient means of identifying potentially pathogenic staphylococci, based on the ability of coagulase-positive species to split DNA. DNases produced by the organisms hydrolyse the DNA molecule to a mixture of smaller mono and poly nucleotides. Perfect correlation between coagulase activity and DN'ase production using S. aureus strains from clinical specimens can be observed with this medium. There are many publications, which have also reported a close correlation.

**Q.C. organisms:** *S. aureus, S. epidermidis*

**Storage**: Plates up to 7 days: at 2-8°C in the dark. Capped container up to 1 month at 4°C in the dark.

**Inoculation**: Use a heavy inoculum on a small area. Four or more organisms can be tested on one 90mm petri dish.

**Incubation**: 37°C aerobically for 18-24 hours.

**Interpretation:**
Having obtained good growth flood the plate with 1N hydrochloric acid. This will precipitate the DNA in the medium. DN'ase producing organisms will be surrounded by a clear area where the DNA has been broken down into fractions, which are not precipitated by the Hydrochloric acid. Gram positive, catalase positive cocci that produce DN'ase can be provisionally classified as S. aureus, and confirmed by tube coagluase or thermostable DN'ase tests. D.N.'ase is also produced by some Gram negative bacilli such as Serratia marcescens, Pseudomonas aeruginosa. Some corynebacteria and streptococci may also produce DN'ase.

**References**
Messinova, O. V., Yusupova, D. V. and Shamsutdinov, N. S. 1963. Deoxyribonuclease activity of Corynebacterium and its relation to virulence. Fed. Proc. 22, T1033.
Streitfeld, M. M., Hoffmann, E. M. and Janklow, H. M. 1962..

**DRIGALSKI LACTOSE AGAR**

A selective medium for the isolation of the Gram-negative bacteria

**Code: KM8302**

| **Typical formula** | **(g/l)** |
|---|---|
| Peptone | 15.0 |
| Beef Extract | 3.0 |
| Yeast Extract | 3.0 |
| Sodium Desoxycholate | 1.0 |
| Sodium Thiosulphale | 1.0 |
| Lactose | 15.0 |
| Agar | 13.0 |
| Crystal Violet | 5 mg |
| Brom Thymol Blue | 80 mg |

pH 7.4 +/- 0.2

**Directions**
Suspend 51g in 1000ml of cold distilled water; heat to boiling, and sterilise by autoclaving at 115 °C for 20 minutes.

**Description and Method**
Drigalski Lactose Agar is a selective medium used for the isolation of the Gram-negative bacteria from urine, faeces and other clinical specimens. The Gram-positive bacteria are inhibited by crystal violet and sodium desoxycholate. The Gram-negative bacteria grow with different characteristics depending on their ability to ferment lactose. Coliform organisms *(E. coli, Klebsiella, Citrobacter, Enterobacter)* ferment lactose with production of acids, and a colour change of bromothymol blue indicator grow with yellow colonies. Gram-negative lactose non fermenting bacteria *(Salmonella, Shigel!a, Proteus, Alkaligenes, Pseudomonas,* etc.) grow with green-blue colonies.

**Quality assurance** (37°C-24 hrs)

Productivity control
*E.coli* ATCC 25922: growth, yellow colonies
*P.mirabilis* ATCC 12453: growth, blue-green colonies
*S.typhimurium* ATCC 14028*:* growth, blue-green colonies

Selectivity control
*E.faecalis ATCC 29212:* inhibited

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

**D.C.L.S. Agar**
**(**Desoxycholate Citrate Lactose Sucrose Agar**)**

A modification of D.C.A. medium for the improved isolation and recognition of *salmonella* and *shigella*.

**Code: KM1025**

| **Typical formula** | **(g/l)** |
|---|---|
| Peptone mix | 10.0 |
| Beef Extract | 5.0 |
| Lactose | 5.0 |
| Sucrose | 5.0 |
| Sodium citrate | 7.5 |
| Sodium thiosulphate | 2.5 |
| Sodium desoxycholate | 2.5 |
| Agar | 12.0 |
| Neutral Red | 0.03 |

pH: 7.2 ± 0.2

**Directions:**
Suspend 50 grams of powder, disperse in 1 litre of deionised water. Allow to stand for 10 minutes then heat gently with frequent mixing and bring to the boil. Simmer for 1 minute to complete dissolution of the solids. Cool to 47ºC then distribute 20ml into Petri dishes. Dry the surface by partial exposure, before use. **Do not** re-melt or autoclave this medium.

**Q.C. organisms:** *Salmonella sp. , E. coli*

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

**Inoculation:** Surface plating, streaking out to single colonies.

**Incubation:** 37ºC aerobically for 24 hours.

**References:**
Hynes, M. J Path Bact. 1942. 54: 193-207
Leifson, E. J Path Bact 1935. 40: 581-589.

**D.C.A. Hynes**
(Desoxycholate Citrate Agar Hynes modification)

**Code: KM1027**

| **Typical formula** | **(g/l)** |
|---|---|
| Balanced Peptone | 5.0 |
| Lactose | 10.0 |
| Sodium thiosulphate | 5.4 |
| Beef Extract | 5.0 |
| Sodium citrate | 8.5 |
| Ferric citrate | 1.0 |
| Sodium desoxycholate | 5.0 |
| Neutral red | 0.02 |
| Agar | 12.0 |

pH: 7.4 ± 0.2

**Directions**
Suspend 52g of powder, disperse in 1 litre of deionised water in a two litre flask. Bring to the boil over gauze, swirling frequently to prevent burning. Simmer for 30 seconds to dissolve. Cool to 47°C before pouring plates. Dry the surface before inoculation. **Do not** re-melt or autoclave this medium.

**Description**
A modification of Leifson's D.C.A. medium. This medium was designed to be more inhibitory to commensal flora whilst allowing for adequate growth of Salmonella spp and Shigella spp. The citrate and desoxycholate levels are significantly increased. To keep the desoxycholate in solution the pH also had to be increased. The medium still uses lactose fermentation and hydrogen sulphide production as differential indicators.

**Appearance:** Pink, clear, bile aggregates may appear on the surface on refrigeration.

**Q.C. organisms**: *Salmonella sp.* & *E. coli*

**Storage:** Plates - up to 7 days at 2-8°C in the dark.

**Inoculation**: Surface, streaking out for single colonies.

**Incubation**: 37°C aerobically for 24 hours.

**DECARBOXYLASE BASE BROTH Falkow**

A base for amino acid decarboxylation tests

**Code: KM5672F**

**Typical formula (g/l)**

| | |
|---|---|
| Peptone | 5.00 |
| Yeast Extract | 3.00 |
| Glucose | 1,00 |
| Brornocresol Purple | 0.02 |

**DECARBOXYLASE BASE BROTH Moeller**

Base for amino acid decarboxylation tests

**Code: KM5672M**

**Typical formula (g/l)**

| | |
|---|---|
| Peptone | 5.00 |
| Beef Extract | 5.00 |
| Pyridoxal | 0.005 |
| Glucose | 0.50 |
| Bromocresol Purple | 0.01 |
| Cresol Red | 0.005 |

pH 6.0 +/- 0.1 (Moeller)
6.8 +/- 0.1 (Falkow)

**Directions**
Suspend 10.5g of Moeller Broth Base or 9g of Falkow Broth Base in 1000ml of cold distilled water heat to dissolve, add L-amino acids in a final concentration of 1% *(w/v);* if necessary readjust the pH. Distribute in 1-1.5 ml amounts in small tubes, seal with sterile liquid paraffin and sterilise at 121°C for 15 minutes.

**Description**
Decarboxylase Base Broth M and Decarboxylase Base Broth F, are the bases used for amino acid decarboxylation tests for the identification of *Enterobacteriaceae*. Arginine, lysine and ornithine are converted to the correspondent amines.

**Method**
The three sets of tubes containing respectively the three amino acids are inoculated with the organism by using a needle, covered with 4-5ml of mineral oil and incubated at 37°C for 4 days. Carry out the relevant checks by using tubes inoculated without the amino acids and daily check the colour change of the medium in all the tubes. Moeller medium contains bromocresol purple and bromocresol red and is violet coloured; Falkow medium contains only bromocresol purple and is purple coloured. Glucose present in both media is fermented by all the enterobacteria with colour change of the indicator system from violet to yellow. The medium, when made acidic, allows the amino acid decarboxylation reactions with formation of amines (cadaverine from lysine, putrescine from arginine

and ornithine). These amines make the medium alkaline and cause a new colour change of the indicator from yellow to violet or purple. The negative reaction is therefore given by the appearance of a yellow colour in the tube, the positive reaction by a violet or purple colour. The following table summarises the reactions given by a few organisms.

| Microorganisms | Lysine | Ornithine | Arginine |
|---|---|---|---|
| *Escherichia coli* | + | *var.* | *var.* |
| **Enterobacter cloacae** | | **+** | **+** |
| *Enterobacter aerogenes* | + | + | - |
| *Edwardsiella tarda* | + | + | - |
| *Salmonella typhi* | + | - | *var.* |
| *Citrotacrer treundi* | - | *var.* | *var.* |
| **Proteus vulgaris** | **-** | **-** | **-** |
| *Proteus mirabilis* | - | + | - |
| *Shigella dysenteriae* | - | | - |
| *Shigella sonnei* | + | - | |
| *Serratia liquefaciens* | *var.* | - | - |
| *Serratia marcescens* | + | + | - |
| *Klebsiella pneumoniae* | - | + | - |

**User quality assurance** (37°C-48 h with mineral oil)

Positive control
Decarboxylase Medium + Lysine*: S.marcescens* ATCC 81100
Decarboxylase Medium + Lysine*: S.marcescens* ATCC 13124
Decarboxylase Medium + Arginine*: E.cloacae* ATCC 13047

Negative control
Decarboxylase Medium + Lysine*: E.cloacae* ATCC 13047
Decarboxylase Medium + Lysine*: K.pneumoniae* ATCC 23357
Decarboxylase Medium + Arginine*: P.mirabilis* ATCC 25933

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

**References**
Cowan and Steel's Manual for Identification of Medical Bacteria, 2nd edition. Cambrige: University Press, 1974.

Moeller V. (1955) - Acta Path. Microbiol. Scand., 36, 158.

**DERMATHOPHYTE TEST MEDIUM**

**Code: KM3912**

Used in tubes for the isolation of dermathophytic fungi

| **Typical formula** | **(g/l)** |
|---|---|
| Peptone | 10.0 |
| Glucose | 40.0 |
| Phenol Red | 0.2 |
| Agar | 12.0 |

pH 5.5 +/- 0.2

**Directions**
Suspend 62g in 1000ml of cold distilled water, heat to boiling and autoclave at 121°C for 10 minutes. Cool to 50°C and aseptically add the contents of one vial of Dermathophyte Supplement reconstituted with 5 ml of sterile distilled water. Mix well and distribute into sterile screw cap bottles or tubes and cool in a slanting position.

**Description**
Dermathophyte Selective Medium is a medium for the isolation of dermathophytic fungi, prepared according to Taplin's formula. Inoculate the medium by surface streaking the specimen to be examined. Incubate at 22-30°C and observe for 7-14 days. Dermathophytic fungi grow with a colour change of the indicator from to violet-red.

**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

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

**References**
Taplin D., Zaias N., Rebbell G., Blank H. (1969) Arch. Derm., 99, 1969.

**DESOXYCHOLATE AGAR**

For the isolation of enteric pathogens and for the enumeration of coliforms in dairy products.

**Code: KM8702**

| **Typical formula** | **(g/l)** |
|---|---|
| Peptone mix | 10.00 |
| Lactose | 10.00 |
| Sodium Chloride | 5.00 |
| Dipotassium Hydrogen Phosphate | 2.00 |
| Ferric citrate | 1.00 |
| Sodium Citrate | 1.00 |
| Sodium Desoxycholate | 1.00 |
| Neutral Red | 0.033 |
| Agar | 15.00 |

pH 7.2 +/- 0.2

**Directions**
Suspend 45g in 1000 ml of cold distilled water, heat to boiling, cool to about 50°C, and distribute into sterile petri dishes. Do not overheat or sterilise in the autoclave.

**Description**
Desoxycholate Agar is a moderately selective medium frequently used in the isolation of enteric pathogens and the enumeration of coliforms in dairy products. The medium can be inoculated with a surface inoculum method as well as a poured plate technique. A thin layer of un-inoculated medium poured over the surface of a galled “pour plate” assists subsequent counting.

**Method**
For the isolation of *Enterobacteriaeae*, it may be used by direct application of the suspect material by means of a loop or swab. For the enumeration of coliforms, the plate is inoculated with the suitably diluted material, which is then covered with a fine layer of the same medium. The coliforms grow as red colonies, whereas non-lactose-fermenting microorganisms, such as *Salmonella* and *Shigella,* are colourless. This medium can be used for the isolation of enteric pathogens in combination with more selective media, such as SS Agar and Brilliant Green Agar.

**User quality assurance** (37°C-24 hrs)

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

Selectivity control
*S.aureus* ATCC 25923: inhibited

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

**D.C.A. Leifson's Agar**
(Desoxycholate Citrate Agar)

For the isolation of intestinal pathogens

**Code: KM1026**

| **Typical formula** | **(g/l)** |
|---|---|
| Peptone | 5.00 |
| Beef Extract | 5.00 |
| Lactose | 10.00 |
| Sodium Citrate | 5.00 |
| Sodium Thiosulfate | 5.00 |
| Ferric Citrate | 1.00 |
| Sodium Desoxycholate | 2.50 |
| Neutral Red | 0.03 |
| Agar | 15.00 |

pH 7.3 +/- 0.2

**Directions**
Suspend 48.5g in 1000ml of cold distilled water, heat to boiling, cool to about 45°C and pour into sterile petri dishes. Dry the agar surface before use. Do not overheat and do not sterilise in the autoclave.

**Description**
Desoxycholate Citrate Agar is a modification of Leifson's medium and is used for the isolation of pathogenic enteric bacteria from clinical samples. Desoxycholate Citrate Agar is less selective than Hynes formulation.

**Method**
Desoxycholate Citrate Agar can be directly heavily inoculated with pathological materials, such as faeces and urine, or it can be used for the subculture from Selenite or other enrichment broths. In the latter case it is better to use a light inoculation of not more than a loopful of broth. *Proteus* is usually inhibited and, if inoculated on dry plates, does not swarm, but forms large mucoid colonies. *E. coli* grows poorly, ferments the lactose and forms red colonies. *Salmonella* and *Shigella* do not ferment the lactose, and form colourless colonies. The Gram-positive bacteria are completely inhibited.

**User quality assurance** (37°C-24hrs)

Productivity control
*S.enteritidis* ATCC 13076: growth, colourless colonies
*S.sonnei* ATCC 25931: growth, colourless colonies

Selectivity control
*E.coli* ATCC 25922: poor growth, red colonies
*S.aureus* ATCC 25923: inhibited

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