PHENYLALANINE AGAR

A differentiation medium for Gram-negative bacilli

Code: KM3161

Typical formula	**(g/l)**
Yeast Extract	3.0
D-L Phenylalanine	2.0
Disodium Phosphate	1.0
Sodium Chloride	5.0
Agar	15.0

pH 7.2 +/- 0.1

Directions

Suspend 26g in 1000ml of cold distilled water, heat to boiling, distribute into test tubes and sterilise by autoclaving at 121°C for 15 minutes.

Description

Phenylalanine Agar is recommended for the differentiation of Gram-negative bacilli based on the ability of the microorganisms to produce phenylpyruvic acid by oxidative deaminisation. Prepared according to a modification of the formula described by Ewing, Davis and Reavis, the medium contains phenylalanine, which, by oxidative deaminisation, produces phenylpyruvic acid, which is detected by the addition to the culture of ferric chloride. It reacts with the by-product to produce a light to deep green colour. *Proteus* spp, *Morganella* spp and *Providencia* spp. deaminate the phenylalanine, whereas the other enteric bacteria do not possess the specific enzyme and, therefore, give a negative reaction.

Method

Streak the slant surface of Phenylalanine Agar with a loopful of pure culture.
Incubated at 35-37°C for 18-24 hours. Following incubation apply 4-5 drops of 10% ferric chloride solution directly to the slant. Gently agitate the tubes and observe for the development of a green colour within 1-5 minutes (positive reaction). Negative results will take on a yellow colour due to the colour of ferric chloride. *Proteus* spp, *Morganella* spp and *Providencia* spp. give a positive reaction, whilst other members of the *Enterobacteriaceae* genus give a negative reaction.

Quality assurance (37°C-24hrs)

Phenylalanine positive control
P.mirabilis ATCC 25933

Phenylalanine negative control
E.coli ATCC 25922

Storage

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

References

Ewing, W.H., Davis B.R. & Reavis, R.W. (1975) - Publ. Health Lab., 15, 153.
Report of the Enterobacteriaceae Subcommittee of the Nomenclature Committee of the International Association of Microbiological Societies (1958) - Int. Bull. Bact. Nam. Tax., 8, 25.
Singer, J. & Volcani, B.E. (1965) - J. Bact., 69, 303.

Plate Count Agar (SP)

A medium equivalent to A.P.H.A. Plate Count Agar and is suitable for the determination of total viable counts in food products by surface count, pour plate and spiral plate methods

Code: KM1085

Typical formula	**(g/l)**
Tryptone	5.0
Yeast Extract	2.5
Glucose	1.0
Agar	12.0

pH: 7.0 ± 0.2

Directions
Suspend 20.5g of powder in 1 litre of deionised water. Allow to stand for 10 minutes, swirl to mix then sterilise by autoclaving at 121ºC for 15 minutes. Cool to 47ºC then pour into petri dishes.

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

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

Inoculation: Surface, or pour plate.

Incubation: 30ºC aerobically for 48 hours for aerobic mesotroph count. 6ºC aerobically for 10 days for aerobic psychrotroph count. 55ºC aerobically for 48 hours for aerobic thermotroph count.

Interpretation: Count all colonies or use spiral plating colony count equipment.

References
American Public Health Association (1985) Standard Methods for the Enumeration of Water and Wastewater. 16th Edition. American Public Health Association Inc.

PLATE COUNT AGAR (Standard Methods)

A medium for the enumeration of aerobic microorganisms and heterotrophic facultative anaerobes in water, food and animal feeding stuff.

Code: KM1084

Typical formula	**(g/l)**
Tryptone	5.0
Yeast Extract	2.5
Glucose	1.0
Agar	15.0

pH 7.0 +/- 0.2

Directions
Suspend 23.5g in 1000ml of cold distilled water, heat to boiling distribute and sterilise by autoclaving at 121°C for 15 minutes. Cool to 45°C and distribute into inoculated sterile petri dishes.

Description
Plate Count Agar (Standard Methods) of the APHA, AOAC, ICMSF, ISO, is the standard medium for the enumeration of aerobic microorganisms and heterotrophic facultative anaerobes in water, food and animal feeding stuff. The technique to be followed when performing the microbial count on the various materials should adhere as closely as possible to the recommendations of the Standard Methods quoted.

Method
The method reported by ISO 4833 (General guidance for the enumeration of microorganisms – colony count technique at 30°C) is the following:

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 recommends the use of peptone salt (see Maximum Recovery Diluent), as general diluent for foods and animal feeding stuffs.
2. Take two sterile petri dishes and transfer, by means of a sterile pipette, to each dish 1ml of the test sample, if the product is liquid, or 1ml of the initial suspension in the case of other products.
3. Take two other sterile petri dishes and transfer, by means of an other sterile pipette, to each dish 1ml of the first decimal dilution (10^{-1}) of the test sample, if the product is liquid, or 1ml of the first decimal dilution (10^{-2}) of the initial suspension in the case of other products.
4. If necessary repeat the procedure with the further dilutions, using a fresh sterile pipette for each decimal dilution.
5. Pour about 15ml of the Agar cooled to 45 °C into each petri dishes.
6. Carefully mix the inoculum with the medium by rotating the plates and allow to solidify the plates on a cool horizontal surface. If it is suspected that the sample contains microorganisms whose colonies will overgrow the surface of the medium, pour about 4ml of sterile water agar medium (agar 12-18g + water 1000ml) on the surface of inoculated medium.
7. Invert the prepared plates and place them in the incubator at 30 °C for 72 hours.
8. Do not stack the dishes more than six high. Stacks of dishes should be separated from one another and from the walls and the top of the incubator.

9. Count the colonies on the plates containing more than 10 and less than 300 colonies.
10. For the expression of the results and the evaluation of precision, repeatability, reproducibility and interpretation of the results see the International Standards quoted.
11. To count microorganisms, which require other growth temperatures, incubate at 5-7°C for 10 days, at 20°C for 3-5 days and at 45°C for 2-3 days.

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

References
AOAC (1995) - Official Methods of Analysis, 16th ed.
APHA (1978) - Standard Methods for the Examination of Dairy Products, 14th ed.
APHA (1985) - Standard Methods for the Examination of Water and Wastewater, 16th ed.
FDA (1995) Bacteriological Analytical Manual, 8th ed. Revision A, 1998. Published by AOAC International.
ISO 4833 Microbiology - General Guidance for the enumeration of micro-organisms. Colony count technique at 30°C. 1991-03-01

POTATO DEXTROSE AGAR

For the detection of yeast and mould in butter and other dairy and food products

Code: KM1086

Typical formula	**(g/l)**
Potato Extract	4.0
Glucose	23.0
Agar	15.0

pH 5.6 +/- 0.2

Directions
Suspend 42g in 1000ml of cold distilled water, heat to boiling and sterilise by autoclaving at 121ºC for 15 minutes. When used as a plating medium for enumerating yeasts and moulds it is often desirable to melt the sterilised medium in boiling water and acidify to pH 3.5 with 1ml of lactic acid 10%. To preserve the solidifying properties of the agar do not heat after addition of the acid.

Description
Potato Dextrose Agar is used to detect and count yeasts and moulds in butter, dairy products and other foods, as specified by the APHA. The medium is commonly used to stimulate mycelium sporulation and to morphologically identify yeasts and moulds. The selectivity of the medium is due to its acid pH (3.5), which inhibits the growth of bacteria.

Method
Prepare dilute emulsions or suspensions of the sample to be tested. Make the poured plates in the usual manner mixing 15ml of medium with 1ml of the serial dilutions. Incubate at 21°C for 5 days. Count the number of yeast and mould colonies.

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

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

References
APHA (1978), Standard Methods or the Examination of Dairy Products, l4th edition
APHA (1985) - Compendium of Methods for the Microbiological Examination of Foods, 2nd ed.

Pseudomonas Agar Base

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

Code: KM1088

Typical formula	**(g/l)**
Acid Casein	10.0
Gelatin Peptone	16.0
Potassium sulphate	10.0
Magnesium chloride	1.4
Agar	11.0

pH: 7.1 +/- 0.2

Directions
Weigh 48.4 grams of powder and disperse in 1 litre of deionised water. Add 10mls of glycerol. Sterilise by autoclaving at 121ºC for 15 minutes. Allow the medium to cool to 47ºC then add the contents of 2 vials of either C.N. supplement or C.F.C. supplement. Mix well and pour into petri dishes.

Description
The base medium is a modification of King's medium A which uses magnesium and potassium salts to enhance production of the pigments pyocyanin (green) and fluorescein (detected by U.V./blue light). The medium is made selective for Pseudomonas aeruginosa by the addition of C.N. supplement. Alternatively the medium can be made selective for Pseudomonas species generally by the addition of C.F.C. supplement.

Quality assurance organisms: *Ps. aeruginosa, E. coli* (inhibition)

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

Inoculation: Surface, spread 0.1 to 0.5mls of sample over entire surface.

Incubation: 25-30ºC aerobically for 48 hours.

Interpretation: Count all colonies as Pseudomonas species. Colonies that exhibit the pyocyanin and fluorescein pigments count as Ps. aeruginosa.

References
King, E. O., Ward, M. K. and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44: 301.
Goto, S. and Enomoto, S. 1970. Jap. J. Microbiol. 14: 65-72.
Mea, G. C. and Adams, B. W. 1977. Br. Poult. Sci. 18: 661-667.

Purple Broth Base

For use with added carbohydrate in differentiating pure cultures, particularly of enteric organisms, based on fermentation reactions.

Code: KM5721

Typical Formula	**g/ltr**
Beef Extract	1.0
Proteose Peptone	10.0
Sodium Chloride	5.0
Bromocresol Purple	0.02

pH 6.8 ± 0.2 at 25ºC

Directions for use

Weigh 16 grams of powder and add to 1 litre of cold deionised water (conductivity <10ms). Sterilise at 121-124°C for 15 minutes.

To prepare fermentation broths, add 0.5-1% carbohydrate before or after sterilisation, depending on heat lability. Dispense into tubes containing inverted fermentation vials.

Palcam Agar Base

An improved selective differential medium for the isolation of Listeria *monocytogenes* from food, clinical and environmental specimens.

Code: KM6148

Typical formula	**(g/l)**
Columbia Peptone Mix	23.0
Aesculin	0.8
Sodium chloride	5.0
Corn Starch	1.0
Yeast Extract	3.0
Glucose	0.5
Mannitol	10.0
Phenol red	0.08
Lithium chloride	15.0
Ferric ammonium citrate	0.5
Agar	12.0

pH: 7.2 ± 0.2

Directions
Weigh 71 grams of powder and disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121ºC. for 15 minutes. Cool to 50ºC and add 2 vials of Supplement. Mix thoroughly before dispersing.

Description
Improved selectivity is achieved by the combination of antibiotic supplements and microaerobic incubation, whilst the double indicator system of aesculin hydrolysis and mannitol fermentation aids differentiation of Listeria spp from enterococci and staphylococci which can be confused with Listeria spp on other types of culture media.

Q.C. organisms: *L. monocytogenes , E. coli* (inhibition)

Storage: Plates up to 7 days at 4ºC. in the dark.

Inoculation: 0.1 ml of sample selectively enriched in Palcam Broth (or other enrichment medium) spread over surface of plate.

Incubation: 30ºC. aerobically or microaerobically for 24-48 hours.

Palcam Broth

A selective differential medium for the enrichment of *Listeria spp*. in food, environmental and clinical samples

Code: KM6144

Typical formula	**(g/l)**
Peptone Mix	23.0
Yeast Extract	5.0
Aesculin	0.8
Peptonised Milk	5.0
Phenol red	0.08
Sodium chloride	5.0
Mannitol	5.0
Ferric ammonium citrate	0.5
Lithium chloride	10.0

pH: 7.2 ± 0.2

Directions
Weigh 54.4 grams of powder and disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix and autoclave at 121ºC for 15 minutes. Cool to 50ºC and add two vials of supplement. Mix well and dispense into sterile tubes or bottles.

Description
This Listeria enrichment media contains an indicator system (aesculin), which will signal the presence of a possible Listeria by a browning/blackening of the broth; the result being the indication of a potential problem up to 48 hours before growth on plating media can be observed.

Q.C. organisms: *L. Monocytogenes , E. coli* (inhibition)

Storage: Capped containers up to 7 days at 4ºC.

Inoculation: Sample or pre-enriched sample added to the broth in the ratio 1: 10.

Incubation: 30ºC for 24 hours and 48 hours.

Subculture: On Palcam Agar. If low numbers of Listeria are present the medium may not produce the brown black colour. All tubes should be sub-cultured onto selective agar before a sample is scored as negative.

PEPTONE AGAR

For use in the presumptive identification of Haemophilus spp.

Code: KM1081

Typical formula	**(g/l)**
Bacteriological peptone	10.0
Sodium chloride	5.0
Agar A	14.0

pH 7.3 +/- 0.2

Directions
Suspend by swirling 29g of powder in 1 litre or the contents of the sachet in the stated volume of distilled or deionised water. Autoclave at 121ºC (15 p.s.i.) for 15 minutes. Mix well before pouring.

Description
The isolation of Haemophilus spp. requires enriched media such as 'chocolate' agars, which provide two factors, required for growth. These two factors termed X and V were later discovered to be haemin and Nicotinamide Adenine Dinucleotide (NAD) respectively. The requirement for X factor, V factor, or both, varies between the species and can be used as a means of presumptive identification. Tests for differentiating Haemophilus spp. on this basis, have mainly involved the use of paper discs impregnated with either or both of the growth factors. To correctly perform such tests a medium free of these factors is required. Peptone Agar is a simple medium containing a carefully chosen peptone free of these factors, but which will support good growth of Haemophilus spp. when these are supplied.

The organism under test is subcultured onto Peptone Agar and X factor, V factor and X + V discs are applied to the surface. After overnight incubation, a clearly defined zone of growth around a particular disc indicates a requirement for that growth factor. (For further details of technique see the appropriate technical leaflet).

Test Species:	Factor X	Factor V	Factor X+V
H.influenzae	-	-	+
H.parainfluenzae	-	+	+
H.ducreyi	+	-	+

+ = growth around the discs.
- = no growth around the discs.

PEPTONE WATER

A liquid medium for indole production by microorganisms

Code: KM1082

Typical formula	**(g/l)**
Tryptone	10.0
Sodium Chloride	5.0

pH 7.3 +/- 0.2

Directions
Suspend 15g in 1000ml of cold distilled water. Mix well to dissolve the liquid medium, distribute into final containers and sterilise at 121°C for 15 minutes.

Description
Peptone Water is recommended for the detection of indole production by microorganisms, especially *Enterobacteriaceae*, because of its high content of tryptophan. The formulation meets the requirement of Tryptone Water recommended by ISO 7251. Organisms possessing the enzyme tryptophanase degrade the tryptophan to produce indole and other metabolic performance. The indole production can be determined by the production of a red-violet colour complex upon application of Kovacs Reagent. If present, indole reacts with the aldehyde group of p-dimethyleminobenzaldehyde with the development of a red-violet colour.

Method
Inoculate a tube containing 5ml of Peptone Water and incubate at 37°C for 24 +/- 3 hours. Incubation at 44°C for 24 hours is advisable for detecting indole production in the confirmation test for faecal coliforms or *E.coli*. After incubation add 1ml of Kovacs Reagent. The formation of a red-violet ring indicates a positive reaction. A yellow-brown ring indicates a negative reaction.

Quality assurance (37°C-24hrs)

Indole positive control
E.coli ATCC 25922

Indole negative control
K.pneumoniae ATCC 23357

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

References
ISO 7251. Microbiology-General Guidance for enumeration of *E. coli* - Most Probable Number Technique. 1993-12-15
Mackenzie, E.F.W., Taylor, E.W. & Gilbert. W.E. (1948) J. Gen. Microbiol., 2, 197.

PEPTONES

Bacteriological Peptone

Code: PH1024

An economical source of nutrients provided by a mixture of meat peptones and tryptone. The growth requirements of most non-demanding organisms will be fulfilled by the range of amino acids, peptides and proteoses in this mixture.

Typical Analysis
Appearance: cream powder
Solubility in water at 5% total
Clarity clear, pale straw colour
pH of 2% solution 7.2 ± 0.2
Total Nitrogen 12% ± 0.5
Amino Nitrogen 5% ± 0.5

Balanced Peptone

Code: PH1004

A rich mixture of tryptone and meat peptones, which fulfils the nutritional demands of a wide variety of microorganisms. This peptone is used a variety culture media formulations.

Typical Analysis
Appearance: beige powder
Solubility in water at 5% total
Clarity clear, pale straw colour
pH of 2% solution 7.2 ± 0.2
Total Nitrogen 12.8% ± 0.5
Amino Nitrogen 5.1% ± 0.5

Mycological Peptone

Code: PH1009

A mixture of peptones with high carbohydrate content for the rapid growth and colonial development of yeasts and moulds. Bacterial growth is inhibited by the low pH of this peptone.

Typical Analysis
Appearance: beige powder
Solubility in water at 5% total
Clarity clear, pale straw colour
pH of 2% solution 5.4 ± 0.5
Total Nitrogen 13% ± 0.5
Amino Nitrogen 1.4% ± 0.5

Proteose Peptone

Code: PH1011

An enzymatic digest of meat modified to encourage the production of toxins by Corynebacterium diphtheriae, staphylococci, Salmonella, and clostridia. This peptone is highly nutritious and suitable for use in culture media for fastidious organisms such as Neisseria, Haemophilus and Pasteurella species.

Typical Analysis
Appearance: cream powder
Solubility in water at 5% total
Clarity clear, light straw colour
pH of 2% solution 7.0 ± 0.2
Total Nitrogen 12.0% ± 0.5
Amino Nitrogen 5.8% ± 0.5

Skim Milk Powder

Code: PH1027

An antibiotic free spray dried skim milk. Used in media for diagnostic tests involving the digestion or coagulation of casein and the fermentation of lactose.

Typical Analysis
Recommended working concentration 10%
Appearance: white powder
Clarity opaque white suspension
Total Nitrogen 5.3% ± 0.5
Lactose 48.0% ± 0.5

Soy Peptone

Code: PH1003

Produced using the enzyme papain to digest soyabean meal, this peptone is a rich source of nutrients with a high carbohydrate content. Most organisms will grow rapidly in this peptone but some bacteria will produce high levels of acid leading to auto-sterilisation unless an adequate buffering system is incorporated.

Typical Analysis
Appearance: cream powder
Solubility in water at 5% total
Clarity clear, straw colour
pH of 2% solution 7.1 ± 0.3
Total Nitrogen 9.0% ± 0.5
Amino Nitrogen 1.6% ± 0.5

Tryptone

Code: PH1005

An enzymatic hydrolysate of casein, rich in peptones and amino acids (including tryptophane). This peptone can be utilised by most bacteria as a growth substrate. This is a pancreatic digest of casein peptone that conforms to the U.S.P. requirements.

Typical Analysis
Appearance: cream powder
Solubility in water at 5% total
Clarity clear, pale straw colour
pH of 2% solution 7.2 ± 0.5
Total Nitrogen 13.0% ± 0.5
Amino Nitrogen 4.9% ± 0.5

Tryptose

Code: PH1008

A blend of peptones suitable for the cultivation of most fastidious organisms including Neisseria gonorrhoeae, Streptococcus milleri and Brucella spp. especially where rapid or profuse growth is required such as in blood culture media and blood agars.

Typical Analysis
Appearance: beige powder
Solubility in water at 5% total
Clarity clear, light straw colour
pH of 2% solution 7.2 ± 0.2
Total Nitrogen 12.5% ± 0.5
Amino Nitrogen 4.9% ± 0.5

PERFRINGENS AGAR BASE

A basal medium used with selective and enrichment supplements for enumeration of *C. perfringens* in foodstuffs.

Code: KM1083

Typical formula	**(g/l)**
Tryptose	15.0
Beef Extract	5.0
Soy Peptone	5.0
Yeast Extract	5.0
Sodium Metabisulphite	1.0
Ferric Ammon.Citrate	1.0
Agar	13.0

Supplemented with:

D-Cycloserine	200 mg
Kanamycin	6 mg
Polymyxin B Sulphate	15.000 IU

Final pH 7.6 +/- 0.2

Directions

Suspend 22.5g of Clostridium Perfringens Agar Base in 500ml of cold distilled water, heat to boiling and autoclave at 121 °C for 15 minutes. For the preparation of Egg Yolk Free Tryptose Sulphite Cycloserine (TSC) Agar add the contents of one vial of D-Cycloserine reconstituted with 5 ml of sterile distilled water. The medium may be prepared also by adding 50ml of Egg Yolk Emulsion to 450 ml of selective medium, cooled to 50°C.

Description

C.perfringens causes gastro enteric disorders characterised by abdominal pain and diarrhoea generally without fever or vomiting. The illness is usually proved by ingestion of inadequately cooled or reheated meats, such as meat pies, stews, gravies, etc, contaminated by soil or faeces. The food poisoning can be diagnosed using quantitative anaerobic cultures to test foods and faeces. The minimum infective dose is 10^5 cells/g of food. For the isolation and enumeration of *C. pertringens* the most widespread methods used in Europe and North America are those of Harmon at al. (Tryptose Sulphite Cycloserine -TSCAgar) and of Shahidi-Ferguson with Kanamycin and Polymyxin B (SFP Agar) with or without Egg Yolk Emulsion. The complete medium TSC Agar, prepared without Egg Yolk Emulsion, meets the requirements given by ISO 7937

Method

For the enumeration of *C. perfringens,* ISO 7937 recommends the following technique:

Prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerning. ISO 6887 recommends the use of peptone salt (see Maximum Recovery Diluent) as general diluent for foods and animal feed stuffs. 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 centres of empty petri dishes. Pour 15 – 20ml of Egg Yolk free TSC Agar into each dish and mix well with the inoculum. When the medium has solidified add an over layer of 10ml of the same TSC Agar. Allow to solidify and incubate in anaerobic jars or other suitable containers at 37°C for 20 hours. Longer

incubation may result in excess blackening along the bottom rim of the plates. Count the black colonies on the plates containing between 15 and 150 characteristic colonies. If parts of the plates are completely blackened count the colonies at the next higher dilution even their number may be less than 15.

To confirm the presence of *C. perfringens* the following tests are recommended:
- reduction of nitrate to nitrite (+)
- motility test (-)
- gelatin liquefaction (+)

Quality assurance (37°C - 24 hrs - Anaer.)

Productivity control
With Supplement: *C.perfringens* ATCC 13124: growth, black green colonies
Without Supplement: *C.sporogenes* ATCC 19404*:* growth, black green colonies

Selectivity control
With Supplement*: E.coli* ATCC 25922 : inhibited

Specificity control
Without*: E.coli* ATCC 25922 : growth, white colonies

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

References
ISO 7937 (1985) Microbiology – General guidance for enumeration of *Clostridium perfringens*- Colony count technique.
Haushild, A.H.W. & Hilaheimer, A. (1974). App. Microbiol. 27, 78
Harmon, S.M., Kautter, O.A. & Peeler, J.T. (1971). App. Microbiol., 22,688
Shehidi, SA. & Ferguson, AR. (1971). App. Microbiol., 21, 500-606

PHENOL RED AGAR BASE

For fermentation reactions of microorganisms

Code: KM3052

Typical formula (g/l)

Peptone mix	11.00
Sodium Chloride	5.00
Phenol Red	0.025
Agar	15.00

PHENOL RED BROTH BASE

For fermentation reactions of microorganisms

Code: KM3102

Typical formula (g/l)

Peptone mix	10.00
Beef Extract	3.00
Sodium Chloride	5.00
Phenol Red	0.018

pH 7.4 +/- 0.2

Directions

Suspend 31g of Phenol Red Agar Base or 18g of Phenol Red Broth Base in 1000ml of cold distilled water. Heat to boiling, distribute the broth into fermentation tubes in amounts of 3-4 ml per tube, and the agar into Erlenmeyer flasks. Autoclave at 121°C 15 minutes. Cool to approximately 50°C and aseptically add a solution of appropriate carbohydrate sterilised by filtration at a final concentration of 1-2 % (w/v). Distribute the agar medium into sterile petri dishes. To reduce preparation time, paper discs containing carbohydrates can be added to the broth in the test tube, or onto the surface of the agar plate.

Description

Phenol Red Agar Base and Phenol Red Broth Base, supplemented with the appropriate carbohydrates, are used to determine the fermentation reactions of microorganisms, especially those that are members of the *Enterobacteriaceae* genus. Both media consist of a sugar free medium base, a pH indicator and a specific carbohydrate or related compound. Phenol Red Media produce acids as a metabolic waste when inoculated with bacteria that are capable of metabolising the substrate. Acid production causes a decrease in pH, which results in a colour shift in the medium.

Method

Pour the sterilised medium into sterile plates (14mm diameter), allow to solidify and then inoculate the surface with a pure culture. For clearer and more rapid results, use the poured plate technique. Place a paper disc on the surface of the inoculated agar, spacing them suitably so that they stick well to the medium (also inoculate a control plate without carbohydrates) and incubate at 37°C. A yellow zone around the discs indicates the production of acid.

Storage

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

References

AOAC (1975) - Methods of Analysis. 12th edition.
APHA (1963) - Diagnostic Procedures and Reagents, 4th edition..
Edwards, P.R. & Ewing W.H. (1972) - Identification of Enterobacteriaceae. 3rd edition. Minneapolis: Burgess Publishing Company.
International Committee on Enterobacteriaceae (1958) - Int. Bull. Bact. Nomencl. Tax., 8, 25.
Sanders, A.C., Faber, J.E. & Cool., T.M. (1967) App. Microbiol., 5, 36.