Phenol Red Agar Base

M053

Intended Use:

A basal medium to which carbohydrates may be added for use in fermentation studies of microorganisms

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
HM peptone B #	1.000
Sodium chloride	5.000
Phenol red	0.025
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Meat extract B

Directions

Suspend 31.02 grams in 1000 ml purified / distilled water. Add 5-10 grams of carbohydrate as desired. Heat to boiling to dissolve the medium completely. Dispense in tubes or flasks as desired and sterilize by autoclaving at 15 lbs pressure (121° C) for 15 minutes. Allow the tubed media to cool in slanted position to form slants with deep butts.

Note : For critical studies, it is recommended to use filter sterilized carbohydrate which is to be incorporated aseptically in sterile medium base

Principle And Interpretation

Phenol Red Agar media are recommended (1,2,5) for studying the fermentation of various carbohydrates individually by the pure cultures of microorganisms.

Proteose peptone and HM Peptone B which is free from fermentable carbohydrates is added in the medium thereby preventing the production of false positive reactions. Phenol Red Agar when supplemented with a specific carbohydrate, a positive carbohydrate fermentation reaction is indicated by the production of a yellow colour in agar due to the effect of acid production. Gas production is indicated by the splitting of agar or by the bubbles formation. Plates or tubes may be incubated aerobically or anaerobically depending on the type of the test organism. Addition of some carbohydrates may result in an acid reaction and hence 0.1N sodium hydroxide can be added dropwise to restore the original colour taking care not to obtain too deep red or cerise colour.

Type of specimen

Pure isolates

Specimen Collection and Handling:

For pure isolate samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Addition of some carbohydrates may result in an acid reaction and hence 0.1N sodium hydroxide can be added dropwise to restore the original colour taking care not to obtain too deep red or cerise colour.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Red coloured clear to slightly opalescent gel forms in tubes as slants

Reaction

Reaction of 3.1% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Organism	Inoculum (CFU)	Growth	without carbohydrate, (Acid)	without carbohydrate, (Gas)	with dextrose, (Acid)	with dextrose, (Gas)
Alcaligenes faecalis ATCC 8750	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Negative reaction, no colour change	Negative reaction
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Proteus vulgaris ATCC 13315	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Shigella flexneri ATCC 12022 (00126*)	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Negative reaction

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Finegold and Baron, 1986, Bailey and Scotts Diagnostic Microbiology, 7th ed., The C.V. Mosby Co., St. Louis.
2. Ewing, 1986, Edwards and Ewings Identification of Enterobacteriaceae, 4th ed., Elsevier Science Publishing Co., Inc., New York.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.

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