



Phenol Red Broth Base

M054

Intended Use:

A basal medium to which carbohydrates are added for determination of fermentation reactions of pure cultures of microorganisms.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
HM peptone B #	1.000
Sodium chloride	5.000
Phenol red	0.018
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Meat extract B

Directions

Suspend 16.02 grams in 1000 ml purified / distilled water, mix well. Heat if necessary to dissolve the medium completely. Mix well and dispense in fermentation tubes (tubes containing inverted Durham's tubes). Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Aseptically add filter sterilized or autoclave sterilized carbohydrate solution to sterile basal medium.

Principle And Interpretation

Phenol Red Broth Medium is formulated as per Vera (2) and is recommended to determine the fermentation reaction of carbohydrates for the differentiation of microorganisms (3, 4, 5). Phenol Red Broth Medium with various added carbohydrates serves as a differential medium by aiding in differentiation of various species and genera by their ability to ferment the specific carbohydrate, with the production of acid or acid and gas (6).

Phenol Red Broth Base is a complete medium without added carbohydrate, which can be used with the addition of 5-10 %, desired carbohydrate. It is used as a negative control for studying fermentations or as a base for the addition of carbohydrates.

Proteose peptone and HM peptone B serve as sources for carbon and nitrogen. Sodium chloride is the osmotic stabilizer. Phenol red is the pH indicator, which turns yellow at acidic pH. Gas formation is seen in Durhams tubes. All of the *Enterobacteriaceae* grow well in this medium. In addition to producing a pH colour shift, the production of mixed acids, notably butyric acids, often results in a pungent, foul odour from the culture medium (1).

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. In addition to producing a pH colour shift, the production of mixed acids, notably butyric acids, often results in a pungent, foul odour from the culture medium (1)

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 coloured homogeneous free flowing powder

Colour and Clarity of prepared medium

Red coloured clear solution without any precipitate

Reaction

Reaction of 1.6% 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.(longer if necessary)

Organism	Inoculum (CFU)	Growth	without carbohydrate, (Acid)	without carbohydrate, (Gas)	with dextrose, (Acid)	with dextrose, (Gas)
<i>Citrobacter freundii</i> ATCC 8090	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
<i>Enterobacter aerogenes</i> ATCC 13048	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
<i>Klebsiella pneumoniae</i> ATCC 13883	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
<i>Proteus vulgaris</i> ATCC 13315	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
<i>Salmonella Typhi</i> ATCC 6539	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
<i>Serratia marcescens</i> ATCC 8100	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
<i>Shigella flexneri</i> ATCC 12022	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. Use before expiry date on the label.

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. Ewing W. H., 1986, Edwards and Ewings Identification of Enterobacteriaceae, 4th ed., Elsevier Science Publishing Co., Inc., New York.
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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. Koneman E. W., Allen S. D., Janda W.M., Schreckenberger P.C., Winn W.C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippincott Company
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification -Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
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8. Vera H. D., 1950, Am. J. Public Health, 40, 1267

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