Phenol Red Broth Base (DM201)

Intended Use
Phenol Red Broth Base (DM201) is recommended for determination of fermentation reactions of microorganisms, when carbohydrates are added.

Product Summary and Explanation
The fermentative properties of bacteria are valuable criteria in their identification.[1, 2] A basal medium for determining the fermentation reactions of microorganisms must be capable of supporting the growth of test organisms and free from fermentable carbohydrates. In 1950, Vera used a fermentation test medium employing the pH indicator phenol red and obtained accurate results.[3] 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.[4-6] Phenol Red Broth Base is a complete medium without added carbohydrate, which can be used with the addition of 5-10%, desired carbohydrate. Some investigators prefer to use 1% rather than 0.5% to ensure against reversion of the reaction due to depletion of the carbohydrate. It is used as a negative control for studying fermentations or as a base for the addition of carbohydrates. Phenol Red Broth Base and Phenol Red Broth with Carbohydrates are referenced in the *Bacteriological Analytical Manual* for the differentiation of *Bacillus* and *Salmonella*.

Principles of the Procedure
Phenol Red Broth Base contains proteose peptone and beef extract which provides the carbon and nitrogen required for good growth of a wide variety of organisms. Sodium chloride maintains the osmotic balance of the medium. Phenol red serves as a pH indicator which turns the color of the medium from red to yellow at acidic pH. If carbohydrate is added the fermentation of carbohydrate turns the pH of medium acidic. 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.

Formula / Liter

Ingredients	Gms / Liter
Proteose peptone	10.00
Beef extract	1.00
Sodium chloride	5.00
Phenol red	0.018
Final pH: 7.4 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 16.02 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Distribute in fermentation tubes (tubes containing inverted Durham's tubes).
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Aseptically add filter sterilized or autoclave sterilized carbohydrate solution to sterile basal medium.

Quality Control Specifications

Dehydrated Appearance	Light yellow to pink homogeneous free flowing powder
Prepared Medium	Red coloured clear solution without any precipitate
Reaction of 1.6% solution	pH 7.4 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours. (Longer if necessary)

Sr. No.	Organisms	Results to be achieved					
		Inoculum (CFU)	Growth	without carbohydrate (Acid)	without carbohy-drate (Gas)	with dextrose (Acid)	with dextrose (Gas)
1.	*Escherichia coli ATCC 25922*	50-100	good-luxuriant	negative reaction, no colour change	negative reaction	positive reaction, Pink colour	positive reaction
2.	*Klebsiella pneumoniae ATCC 13883*	50-100	good-luxuriant	negative reaction, no colour change	negative reaction	positive reaction, Pink colour	positive reaction
3.	*Proteus vulgaris ATCC 13315*	50-100	good-luxuriant	negative reaction, no colour change	negative reaction	positive reaction, Pink colour	positive reaction
4.	*Salmonella Typhi ATCC 6539*	50-100	good-luxuriant	negative reaction, no colour change	negative reaction	positive reaction, Pink colour	positive reaction
5.	*Salmonella Typhimurium ATCC 14028*	50-100	good-luxuriant	negative reaction, no colour change	negative reaction	positive reaction, Pink colour	positive reaction
6.	*Shigella sonnei ATCC 25931*	50-100	good-luxuriant	negative reaction, no colour change	negative reaction	positive reaction, Pink colour	negative reaction
7.	*Shigella flexneri ATCC 12022*	50-100	good-luxuriant	negative reaction, no colour change	negative reaction	positive reaction, Pink colour	negative reaction

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Using a heavy inoculum, inoculate tubes of media with growth from an 18- to 24-hour old pure culture using an inoculating loop.
2. Incubate tubes with loosened caps at 35 - 37°C for 18-24 hours either in an aerobic or anaerobic atmosphere depending on the organism being evaluated.

Results

1. If supplemented with carbohydrate a yellow color in the medium indicates a positive reaction for carbohydrate fermentation.
2. If a Durham tube is used, bubbles in the inverted tube is an indication of gas production.
3. The presence of a single bubble is recorded as positive for the production of gas.
4. Refer to appropriate references for typical reactions produced by various microbial species.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. The addition of some carbohydrates to the basal medium may result in an acid reaction.
2. To ensure accuracy of interpretation, uninoculated control tubes and/or inoculated Phenol Red Broth Base control tubes should be run in parallel with the fermentation tests.

Packaging

Product Name : Phenol Red Broth Base

Product Code : DM201

Available Pack sizes : 100gm / 500gm

References

1. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed., Lippincott Williams & Wilkins, Baltimore, Md.
2. Forbes, Sahm and Weissfeld. 2007. Diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
3. Vera. 1950. Am. J. Public Health, 40:1267.
4. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
5. Becton, Dickinson and Co. 2007. BBL quality control and product information manual for plated and tubed media, BD Diagnostics, Sparks, Md.™
6. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsvier Science Publishing Co., New York, N.Y.

Further Information

For further information please contact your local MICROMASTER Representative.

MICROMASTER LABORATORIES PRIVATE LIMITED
Unit 38/39, Kalpataru Industrial Estate,
Off G.B. Road, Near 'R-Mall', Thane (W) – 400607. M.S. INDIA.
Ph: +91-9320126789/9833630009/9819991103
Email: sales@micromasterlab.com

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