BBL™ Phenol Red Broth Base, 8 mL
BBL™ Phenol Red Broth with Dextrose and Durham Tube
BBL™ Phenol Red Broth with Xylose and Durham Tube

L007486 • Rev. 11 • January 2015

QUALITY CONTROL PROCEDURES

I INTRODUCTION

Phenol Red Broth Base, when supplemented with an appropriate carbohydrate, is used to determine the fermentation activities of microorganisms.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Inoculate the tubes with growth from 18- to 24-h **Trypticase™** Soy Agar with 5% Sheep Blood plate cultures, using a 0.01 mL calibrated loop.
   b. Incubate tubes with loosened caps at 35 ± 2 °C in an aerobic atmosphere.
2. Examine tubes after 18–24 and 42–48 h for growth and reaction. Gas production is defined as the presence of gas in the inverted Durham tube with a corresponding effervescence produced when the tube is gently shaken.
3. Expected Results

Organisms	ATCC™	Phenol Red Broth Base	Dextrose	Xylose
Escherichia coli	25922	N/A	Acid,Gas	Acid, Gas
Klebsiella pneumoniae	33495*	Alkaline	N/A	N/A
Morganella morganii	8019	N/A	N/A	Alkaline
Proteus vulgaris	8427	Alkaline	N/A	N/A
Pseudomonas aeruginosa	10145	N/A	Alkaline	N/A
Salmonella typhimurium	14028**	N/A	N/A	Acid, Gas
Salmonella paratyphi	9150***	N/A	N/A	Alkaline
Shigella flexneri	9199	N/A	Acid	N/A
Streptococcus pneumoniae	6303	Alkaline	N/A	N/A

* *K. pneumoniae* subsp. *pneumoniae*
** *S. choleraesuis* subsp. *choleraesuis* serotype Typhimurium
*** *S. choleraesuis* subsp. *choleraesuis* serotype Paratyphi A

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under “Product Deterioration.”
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.4 ± 0.2.
4. Incubate uninoculated representative tubes at 20–25 °C and 30–35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Phenol Red Broth Base and Phenol Red Broth with carbohydrates are used for the determination of fermentation reactions in the differentiation of microorganisms.

V SUMMARY AND EXPLANATION

The ability of an organism to ferment a specific carbohydrate incorporated in a basal medium, resulting in the production of acid or acid and gas, has been used to characterize a specific species or group of bacteria, aid in the differentiation between genera, and aid in species differentiation.[1,2]

In 1950, Vera recommended using pancreatic digest of casein in fermentation test media.[3] She found that casein peptone could be used with the pH indicator phenol red in fermentation tests with a high degree of accuracy.

VI PRINCIPLES OF THE PROCEDURE

Phenol Red Broth Base is a complete medium without added carbohydrate. It is used as a negative control for fermentation studies or as a base for the addition of carbohydrates by the aseptic addition of **BBL™ Taxo™** Carbohydrate Discs. Pancreatic digest of casein provides nutrients and is low in fermentable carbohydrate.[3] The pH indicator, phenol red, is used to detect acid production.

Phenol Red Broths, prepared with a final concentration of one-half percent carbohydrate, are convenient for the determination of fermentation reactions. Most of the end products of carbohydrate fermentation are organic acids which, in the presence of phenol red, produce a color change in the medium from red to yellow.[1] If gas is produced during the fermentation reaction, it is collected in the inverted Durham tube.

No yellow color should occur in the control tube. If it does, the results cannot be correctly interpreted since acid has been produced without fermentation.

VII REAGENTS

Phenol Red Broth Base

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	10.0	g
Sodium Chloride	5.0	g
Phenol Red	0.018	g

*Adjusted and/or supplemented as required to meet performance criteria.

Phenol Red Broth with carbohydrates contain the above ingredients with, per liter, 5.0 g of the specified carbohydrate.

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[2,4] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Phenol Red Broth Base, 8 mL or Phenol Red Broth with Dextrose and Durham Tube or Phenol Red Broth with Xylose and Durham Tube.

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

If **Taxo** Carbohydrate Discs are being used with tubes of Phenol Red Broth Base, aseptically add the appropriate disc to the tubes prior to inoculation.

Using a heavy inoculum, inoculate tubes of media with growth from an 18- to 24-h pure culture using an inoculating loop. Incubate tubes with loosened caps at 35 ± 2 °C for 18–48 h either in an aerobic or anaerobic atmosphere depending on the organism being evaluated. Incubation up to 30 days may be necessary for a negative result.

User Quality Control: See “Quality Control Procedures.”

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory’s standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Examine the unsupplemented tubes at intervals during the incubation process for growth. If supplemented with carbohydrate, observe for the presence of acid (yellow color) and gas (as evidenced by displacement of the liquid in the Durham tubes). Consult appropriate references for typical reactions produced by various microbial species.[1,2,4-6]

XI LIMITATIONS OF THE PROCEDURE

The use of Phenol Red Broths containing carbohydrates aids in microbial differentiation. Additional biochemical tests, as well as morphological characteristics and serological typing, may be required for identification. Appropriate texts should be consulted for additional information.[2,4-6]

XII PERFORMANCE CHARACTERISTICS

Phenol Red Broth with Dextrose

Prior to release, all lots of Phenol Red Broth with Dextrose are tested for performance characteristics. Representative samples of the lot are directly inoculated with cultures of *Shigella flexneri* ATCC 9199, *Pseudomonas aeruginosa* ATCC 10145 and *Escherichia coli* ATCC 25922 grown 18–24 h on **BBL™ Trypticase™** Soy Agar with 5% Sheep Blood. Tubes are incubated with loose caps at 35–37 °C for 2 days in an aerobic atmosphere. Acid (color change from red to yellow) and gas production is observed with *E. coli*. Acid only is observed with *S. flexneri*. No reaction is observed with *P. aeruginosa*.

Phenol Red Broth with Xylose

Prior to release, all lots of Phenol Red Broth with Xylose are tested for performance characteristics. Representative samples of the lot are directly inoculated with cultures of *Morganella morganii* ATCC 8019, *Salmonella choleraesuis* (subsp. *choleraesuis* serotype Typhimurium) ATCC 14028, *S. choleraesuis* (subsp. *choleraesuis* serotype Paratyphi A) ATCC 9150 and *Escherichia coli* ATCC 25922 grown 18–24 h on **BBL Trypticase** Soy Agar with 5% Sheep Blood. Tubes are incubated with loose caps at 35–37 °C for 2 days in an aerobic atmosphere. Acid (color change from red to yellow) and gas production is observed with *E. coli* and *S. typhimurium*. No reaction is observed with *M. morganii* and *S. paratyphi* A.

Phenol Red Broth Base

Prior to release, all lots of Phenol Red Broth Base are tested for performance characteristics. Representative samples of the lot are directly inoculated with cultures of *Klebsiella pneumoniae* ATCC 33495, *Proteus vulgaris* ATCC 8427 and *Streptococcus pneumoniae* ATCC 6303 grown 18–24 h on **BBL Trypticase** Soy Agar with 5% Sheep Blood. Tubes are incubated with loose caps at 35–37 °C for 2 days in an aerobic atmosphere and examined for any change in color. All organisms produce an alkaline reaction (no color change).

XIII AVAILABILITY

Cat. No.	Description
221897	**BD BBL™** Phenol Red Broth Base, 8 mL, Pkg. of 10 size K tubes
221677	**BD BBL™** Phenol Red Broth with Dextrose and Durham Tube, Pkg. of 10 size K tubes CE
221705	**BD BBL™** Phenol Red Broth with Xylose and Durham Tube, Pkg. of 10 size K tubes CE

XIV REFERENCES

1. MacFaddin, J.F. 2000. Biochemical tests for identification of medical bacteria, 3rd ed., Lippincott Williams & Wilkins, Baltimore.
2. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
3. Vera, H.D., 1950. Relation of peptones and other culture media ingredients to accuracy of fermentation tests. Am. J. Public Health, *40*:1267-1272.
4. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
5. Ewing, W.H. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*. 4th ed. Elsevier Science Publishing Co., New York.
6. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.

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