



BBL™ Phenol Red Broth Base

BBL™ Phenol Red Broth with Adonitol and Durham Tube
BBL™ Phenol Red Broth with Arabinose and Durham Tube
BBL™ Phenol Red Broth with Cellobiose and Durham Tube
BBL™ Phenol Red Broth with Dulcitol and Durham Tube
BBL™ Phenol Red Broth with Glycerol and Durham Tube
BBL™ Phenol Red Broth with Inositol and Durham Tube
BBL™ Phenol Red Broth with Inulin and Durham Tube
BBL™ Phenol Red Broth with Lactose and Durham Tube
BBL™ Phenol Red Broth with Maltose and Durham Tube
BBL™ Phenol Red Broth with Mannitol and Durham Tube
BBL™ Phenol Red Broth with Raffinose and Durham Tube
BBL™ Phenol Red Broth with Rhamnose and Durham Tube
BBL™ Phenol Red Broth with Salicin and Durham Tube
BBL™ Phenol Red Broth with Sorbitol and Durham Tube
BBL™ Phenol Red Broth with Sucrose and Durham Tube
BBL™ Phenol Red Broth with Trehalose and Durham Tube

L009481 • Rev. 00 • January 2006

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 $35 \pm 2^\circ\text{C}$ 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 (See Figure 1 on the following page)

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 7.3 ± 0.2 for Phenol Red Broth with Inulin and 7.4 ± 0.2 for all remaining broths.
4. Incubate uninoculated representative tubes at $20\text{--}25^\circ\text{C}$ and $30\text{--}35^\circ\text{C}$ and examine after 5 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.

Figure 1: Expected Results

	Phenol Red Broth Base	Adonitol	Arabinose	Cellobiose	Dulcitol	Glycerol	Inositol	Inulin	Lactose	Maltose	Mannitol	Raffinose	Rhamnose	Salicin	Sorbitol	Sucrose	Trehalose
<i>Streptococcus pneumoniae</i> ATCC™ 6305	K							A									
<i>Enterobacter aerogenes</i> ATCC 13048		AG	AG			AG	A				AG	A(G)		AG			
<i>Enterobacter cloacae</i> ATCC 13047				AG	K				AG				AG			AG	
<i>Hafnia alvei</i> ATCC 11604												K	AG				
<i>Escherichia coli</i> ATCC 25922					AG					AG					AG		AG
<i>Klebsiella pneumoniae</i> ATCC 33495*	K	AG	AG	AG			AG		AG	AG		AG		AG	AG		
<i>Morganella morganii</i> ATCC 8019		K		K	K				K	K	K				K	K	K
<i>Proteus vulgaris</i> ATCC 8427	K										K					AG	
<i>Salmonella typhimurium</i> ATCC 14028**									K								K
<i>Salmonella typhimurium</i> ATCC 13311**					A												
<i>Serratia marcescens</i> ATCC 13880			K									K	K				
<i>Shigella flexneri</i> ATCC 9199						K	K							K			
<i>Enterococcus faecalis</i> ATCC 29212						A		K									(A)
<i>Edwardsiella tarda</i> ATCC 15947		K	K								K			K	K		

Key: A = Acid K = Alkaline (A) = late (after 48 h) G = Gas (G) = Variable Gas

* *K. pneumoniae* subsp. *pneumoniae*

** *S. choleraesuis* subsp. *choleraesuis* serotype Typhimurium

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 Casein10.0 g

Sodium Chloride5.0 g

Phenol Red0.018 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

This product is not intended for use directly with specimens or mixed cultures. The organism to be tested must first be in pure culture.

IX PROCEDURE

Material Provided: Phenol Red Broth Base or Phenol Red Broth with Durham Tube and Adonitol or Arabinose or Cellobiose or Dulcitol or Glycerol or Inositol or Inulin or Lactose or Maltose or Mannitol or Raffinose or Rhamnose or Salicin or Sorbitol or Sucrose or Trehalose.

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 (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

A single electrode of sufficiently small size to fit into the tubes should be used to determine the pH potentiometrically of tubed media. The tip of the electrode should be placed below the surface of broth media.

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.^{1,2,4-7}

XI LIMITATION OF THE PROCEDURE

The use of Phenol Red Broths containing carbohydrates aids in microbial differentiation. For identification, organisms must be in pure culture. Additional biochemical tests, as well as morphological characteristics and serological typing, may be required for identification. Appropriate texts should be consulted for additional information.^{1,2,4-7}

XII AVAILABILITY

Cat. No.	Description
221897	BBL™ Phenol Red Broth Base, Pkg. of 10 size K tubes
221671	BBL™ Phenol Red Broth with Adonitol and Durham Tube, Pkg. of 10 size K tubes
221673	BBL™ Phenol Red Broth with Arabinose and Durham Tube, Pkg. of 10 size K tubes
221675	BBL™ Phenol Red Broth with Cellobiose and Durham Tube, Pkg. of 10 size K tubes
221679	BBL™ Phenol Red Broth with Dulcitol and Durham Tube, Pkg. of 10 size K tubes
221681	BBL™ Phenol Red Broth with Glycerol and Durham Tube, Pkg. of 10 size K tubes
221683	BBL™ Phenol Red Broth with Inositol and Durham Tube, Pkg. of 10 size K tubes
221685	BBL™ Phenol Red Broth with Inulin and Durham Tube, Pkg. of 10 size K tubes.
221687	BBL™ Phenol Red Broth with Lactose and Durham Tube, Pkg. of 10 size K tubes
221689	BBL™ Phenol Red Broth with Maltose and Durham Tube, Pkg. of 10 size K tubes
221691	BBL™ Phenol Red Broth with Mannitol and Durham Tube, Pkg. of 10 size K tubes
221693	BBL™ Phenol Red Broth with Raffinose and Durham Tube, Pkg. of 10 size K tubes
221695	BBL™ Phenol Red Broth with Rhamnose and Durham Tube, Pkg. pf 10 size K tubes
221697	BBL™ Phenol Red Broth with Salicin and Durham Tube, Pkg. of 10 size K tubes
221699	BBL™ Phenol Red Broth with Sorbitol and Durham Tube, Pkg. of 10 size K tubes
221701	BBL™ Phenol Red Broth with Sucrose and Durham Tube, Pkg. of 10 size K tubes
221703	BBL™ Phenol Red Broth with Trehalose and Durham Tube, Pkg. of 10 size K tubes

XIII REFERENCES

1. MacFaddin, J.F. 2000. Biochemical tests for identification of medical bacteria, 3rd ed., Lippincott Williams & Wilkins, Baltimore.
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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. Tenover (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.
7. Isenberg, H.D. (ed.). 2004. Clinical microbiology procedures handbook, vol. 1, 2 and 3, 2nd ed. American Society for Microbiology, Washington, D.C.

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