BBL™ Phenylalanine Agar Slants

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Phenylalanine Agar is a medium for the differentiation of enteric bacilli on the basis of their ability to produce phenylpyruvic acid by oxidative deamination.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Using a 0.01 mL calibrated loop, inoculate the agar surfaces with 10^{-1} dilutions of 18- to 24-h **Trypticase™** Soy Broth cultures.
   b. Incubate tubes with loosened caps at 35 ± 2 °C in an aerobic atmosphere.
2. Examine tubes after 18 – 24 h for growth.
3. Add five drops of 10% aqueous ferric chloride solution to each tube and observe for the production of a dark green color (positive reaction).
4. Expected Results

Organisms	ATCC®	Reaction
**Proteus vulgaris*	8427	Positive (green color)
Morganella morganii	8019	Positive (green color)
Providencia rustigianii	12013	Positive (green color)
**Escherichia coli*	25922	Negative (no color change)

*Recommended organism strain for User Quality Control.

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. 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

Phenylalanine Agar is used for the differentiation of enteric bacilli on the basis of their ability to produce phenylpyruvic acid by oxidative deamination.

V SUMMARY AND EXPLANATION

Henrickson initially demonstrated that *Proteus* species were able to transform phenylalanine to phenylpyruvic acid.[1] Singer and Volcani,[2] Hamida and LeMinor[3] and others studied the reaction and emphasized its usefulness in the taxonomy of the *Enterobacteriaceae*.

Buttiaux et al. developed a culture medium containing phenylalanine in their study of the characteristic biochemical properties of the *Proteus* and *Providencia* genera.[4] This medium was designed to differentiate members of the *Proteeae* from other members of the *Enterobacteriaceae* by the ability of organisms in the genera within the *Proteeae* to deaminate phenylalanine to phenylpyruvic acid by enzymatic activity.[5] *Proteus*, *Providencia* and *Morganella* species possess this capability. **BBL** Phenylalanine Agar is a modification of the original formulation of Ewing et al.[6]

VI PRINCIPLES OF THE PROCEDURE

The phenylalanine serves as the substrate for enzymes which are able to deaminate it to form phenylpyruvic acid. The addition of four or five drops of a 10% aqueous ferric chloride solution (or a 12% aqueous ferric chloride solution acidified with 2.5 mL of concentrated HCl per 100 mL of reagent) to the cultures following incubation results in the appearance of a light to deep green color (positive reaction) or no color change (negative reaction).

VII REAGENTS

Phenylalanine Agar

Approximate Formula* Per Liter Purified Water

DL-Phenylalanine	2.0 g
Yeast Extract	3.0 g
Sodium Chloride	5.0 g
Sodium Phosphate	1.0 g
Agar	12.0 g

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

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.[7,8] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Phenylalanine Agar Slants

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

Test Procedure: Observe aseptic techniques.

Using a heavy inoculum, inoculate tubed slants with growth from an 18- to 24-h pure culture. Incubate tubes aerobically at 35 ± 2 °C for 4 h or 18 – 24 h. If the inoculum is sufficiently heavy, a 4 h incubation period should be adequate.[5]

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

Following the incubation period, add four or five drops of the ferric chloride reagent to the slants. Gently rotate the tube to loosen the growth. Observe for the production of a green color (positive reaction) within 1 – 5 min.

Members of the *Proteus*, *Morganella* and *Providencia* genera produce positive results. Other genera within the *Enterobacteriaceae* are negative for phenylpyruvic acid production.[9,10]

XI LIMITATIONS OF THE PROCEDURE

A positive reaction must be interpreted within the first 5 min following addition of the reagent as the green color fades rapidly.

A few other strains of *Enterobacteriaceae* are also phenylalanine positive: *Enterobacter agglomerans* (20%), *Enterobacter sakazakii* (50%), *Rahnella aquatilis* (95%), and *Tatumella ptyseos* (90%).[10]

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[7-9]

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Phenylalanine Agar slants are tested for performance characteristics. Using a 0.01 mL calibrated loop, representative samples of the lot are streak-inoculated with **Trypticase** Soy Broth cultures diluted 10^{-1} of *Escherichia coli* (ATCC 25922), *Morganella morganii* (ATCC 8019), *Proteus vulgaris* (ATCC 8427) and *Providencia rustigianii* (ATCC 12013). The tubes are incubated with loosened caps at 35 ± 2 °C. After 18 – 24 h incubation, the slants are observed for the amount of growth. All cultures exhibit moderate to heavy growth. Subsequently, 4 – 5 drops of a 10% aqueous solution of Ferric Chloride are added to each tube. The tube is gently rotated to loosen growth. Within 1 – 5 min, the slants inoculated with *M. morganii*, *P. vulgaris* and *P. rustigianii* produce an intense green color (positive reaction) indicating the presence of phenylpyruvic acid in the medium. No reaction (no color change) occurs with the slant inoculated with *E. coli*.

XIII AVAILABILITY

Cat. No.	Description
221342	**BD BBL™** Phenylalanine Agar Slants, Pkg. of 10 size K tubes

XIV REFERENCES

1. Henrikson, S.D. 1950. A comparison of the phenylpyruvic acid reaction and the urease test in the differentiation of *Proteus* from other enteric organisms. J. Bacteriol. *60*:225-231.
2. Singer, J., and B.E. Volcani. 1955. An improved ferric chloride test for differentiating *Proteus-Providencia* group from other *Enterobacteriaceae*. J. Bacteriol. *69*:303-306.
3. Hamida, F.B., and L. LeMinor. 1956. Une methode rapide de recherche de la transformation de la L-phenylalanine en acide phenyl-pyruvique. Ann. Inst. Pasteur. *90*:671-673.
4. Buttiaux, R., R. Osteux, R. Fresnoy, and J. Moriamez. 1954. Les proprietes biochemiques caracteristiques du genre *Proteus*. Inclusion souhaitable des *Providencia* dans celuici. Ann. Inst. Pasteur Lille. *87*:375-386.
5. MacFaddin, J.F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore.
6. Ewing, W.H., B.R. Davis and R.W. Reavis. 1957. Phenylalanine and malonate media and their uses in enteric bacteriology. Public Health Lab. *15*:153.
7. 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.
8. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
9. 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.
10. Farmer, J.J., III. 1999. *Enterobacteriaceae*: introduction and identification, p. 442-458. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

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