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
<table>
<thead>
<tr>
<th>Organisms</th>
<th>ATCC®</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>8427</td>
<td>Positive (green color)</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>8019</td>
<td>Positive (green color)</td>
</tr>
<tr>
<td>Providencia rustigianii</td>
<td>12013</td>
<td>Positive (green color)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922</td>
<td>Negative (no color change)</td>
</tr>
</tbody>
</table>

*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.

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 *Proteaeae* from other members of the *Enterobacteriaceae* by the ability of organisms in the genera within the *Proteaeae* 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. 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.

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.

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%).

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.

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. 221342 BD BBL™ Phenylalanine Agar Slants, Pkg. of 10 size K tubes

XIV REFERENCES

Technical Information: In the United States, contact BD Technical Service and Support at 800-638-8663 or www.bd.com/ds.