PROVIDENCE Sacred Heart Medical Center & Children's Hospital

I. Purpose and Test Principle

Most strains of *Pseudomonas aeruginosa* produce a pigment called pyocyanin. *P. aeruginosa* is the only known *Pseudomonas* species to produce this pigment. Pseudomonas P agar was developed to enhance the production of pyocyanin. This medium contains enzymatic digest of gelatin to provide amino acids and other essential nitrogenous substances. The gelatin peptone used is low in phosphorous to minimize the inhibitory action on pyocyanin. Magnesium, potassium, and sulfate ions incorporated into the medium promote pigment production.

Pseudomonas P agar may also be used for temperature tolerance testing at 42°C. The ability to grow at this temperature is useful for distinguishing *P. aeruginosa* and several other glucose non-fermenting gram-negative rods.

II. Specimen Information

The organism to be tested must be in pure culture.

III. Reagents & Equipment

- Remel Pseudomonas P Agar Slant
  Storage: On receipt, store media at 2-8°C. Avoid freezing or overheating. Tubes should be allowed to equilibrate to room temperature prior to inoculation.
- Inoculation needle and Bunsen burner
- 42°C incubator

IV. Procedure

A. Using a sterile inoculating loop or needle, sample a well-isolated colony of the test isolate.
B. Lightly inoculate the surface of the agar slant.
C. Cap the tube loosely.
D. Incubate for 18 to 24 h at 42°C. Slow growing organisms may require additional incubation prior to interpreting the results.
E. If growth, but no pigment, appears after incubation at 42°C, the slant can be held at room temperature for up to 7 days and monitored for pigment production.

V. Interpretation of Results

After 18 to 24 h of incubation, examine the slant for growth. If the test isolate demonstrates slow growth on other routine media, the slant should be re-incubated at 42°C to allow sufficient time for the test to be valid.

A. 42°C Temperature Tolerance
   1. If growth is observed, document as positive for growth at 42°C.
   2. If no growth is observed on the slant, document as negative.

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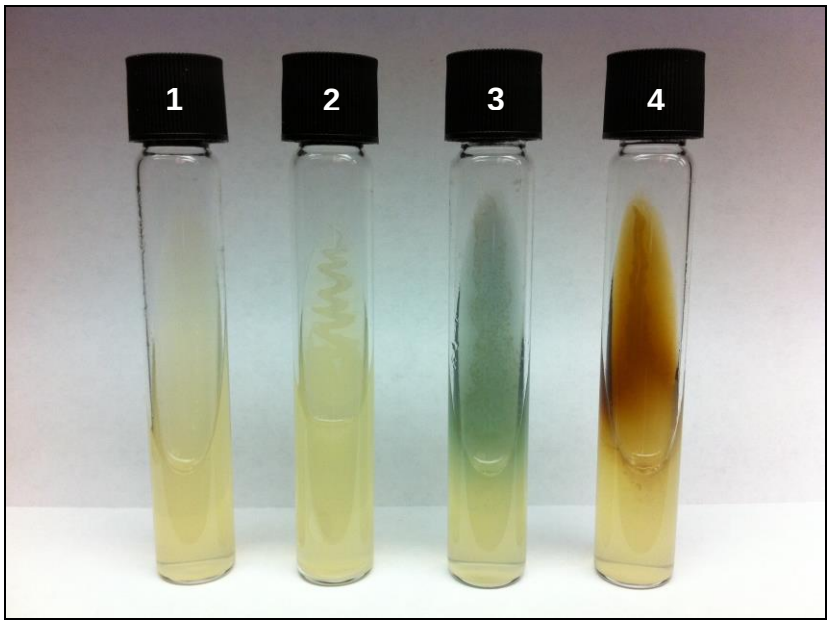
B. Pigment Production

1. If the slant is developing a blue-green pigment, document as positive for pyocyanin. Some *P. aeruginosa* isolates may produce other pigments, such as the brown-black pyomelanin or the red pyorubin.
2. If no pigment is observed, slant may be held at room temperature for up to 7 d if necessary. If no pigment develops, document the isolate as negative for pyocyanin.



Tube 1: No growth at 42°C
Tube 2: Growth at 42°C without pigment production
Tube 3: Growth at 42°C with pyocyanin production
Tube 4: Growth at 42°C with pyomelanin production

VI. Quality Control

Each new lot or shipment of media should be examined for product deterioration and tested with the following control strains by following the procedure outlined above. Incubate tubes at 42°C.

Control strain	Expected Results
P. aeruginosa ATCC 27853	Growth with blue-green pigment
B. cepacia ATCC 17765	Growth with no pigment

VII. Limitations

Not all *P. aeruginosa* isolates produce pigments, especially those from CF patients.

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VIII. Verification of Test Method

A total of 19 isolates were used to evaluate the performance of BBL™ Tech Agar. This included *P. aeruginosa* ATCC 27853 and 11 clinical *P. aeruginosa* isolates, 7 of which were from cystic fibrosis (CF) patients. The remaining isolates included 3 *Pseudomonas putida*, 2 *Pseudomonas fluorescens*, 1 *Alcaligenes xylosoxidans*, and *Burkholderia cepacia* ATCC 17765. Slants were inoculated following the procedure outlined above and incubated at 42°C. The slants were examined at 24 and 48 h. All 12 (100%) of the *P. aeruginosa* isolates grew at 24 h. Seven (58%) of the *P. aeruginosa* isolates produced pigment. Five strains produced pyocyanin (blue-green), 1 strain produced pyomelanin (brown), and 1 strain produced both pyocyanin and pyorubin (blue-green and red). The remaining 5 *P. aeruginosa* isolates did not produce pigment. All of the non-pigmented strains were CF isolates. This is consistent with published data. None of the *P. fluorescens* or *P. putida* isolates grew at 42°C after 48 h of incubation. The *A. xylosoxidans* and *B. cepacia* strains both grew at 42°C but did not produce pigment.

In 2012, BD discontinued production of Tech Agar. The Pseudomonas P Agar Slant produced by Remel was determined to be an equivalent substitute product.

IX. References

A. Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.

B. Package insert: BBL™ Prepared Media for Differentiation of *Pseudomonas* species 88-0647-1, Revised January 1999.

C. Package insert: Remel Pseudomonas P Agar, IFU 1714, Revised October 11, 2010.

D. Pichardo Reyes, E.A., Bale, M.J., Cannon, W.H., Matsen, J. 1981. Identification of Pseudomonas aeruginosa by Pyocyanin Production on Tech Agar. J. Clin. Microbiol. p. 456-458 Vol. 13, No. 3.

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Updates and Revisions: