PSEUDOMONAS P AGAR

INTENDED USE
Remel Pseudomonas P Agar is a solid medium recommended for use in qualitative procedures for differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* spp. based on pyocyanin production.

SUMMARY AND EXPLANATION
In 1954, King et al. developed two media formulations (Medium A and Medium B) to enhance pigment production in pseudomonads.[1] Previous work had demonstrated amino acids, inorganic ions, minerals, and peptones influenced the production of pyocyanin and fluorescein. King determined the production of these pigments was affected by the composition of the peptone in the medium. Pigment production was also influenced by the absence or minimal concentrations of components which may have a detrimental effect. A combination of magnesium chloride and potassium sulfate has been found to most effectively stimulate the production of pyocyanin.[2]

PRINCIPLE
P. aeruginosa is the only species of bacteria known to produce pyocyanin, a blue-green pigment which diffuses into the agar surrounding the growth. Gelatin and meat peptones provide the nutrients necessary for bacterial growth. These peptones contain less than 0.1% phosphorous which has been found to increase pyocyanin production. Magnesium chloride and potassium sulfate in a total amount that does not exceed 2% stimulates pyocyanin production. Glycerol is an energy source.

REAGENTS (CLASSICAL FORMULA)*

Gelatin Peptone	10.0	g	Magnesium Chloride	1.4	g
Meat Peptone	10.0	g	Glycerol	10.0	ml
Potassium Sulfate	10.0	g	Agar	15.0	g
			Demineralized Water	1000.0	ml

pH 7.2 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. The test isolate should be in pure culture and 18-24 hours old. A nonselective or selective agar plate may be used for inoculation of the test isolate.
2. Select the center of a well-isolated colony and streak onto Pseudomonas P Agar.
3. Incubate the plate in ambient air (caps loosened for slants) at 33-37°C for 18-24 hours.
4. Examine the medium for blue-green pigment diffusing into the agar surrounding the growth. If no pigment has developed, incubate at 25°C for an additional 24 hours. If no growth appears, reincubate at 25°C for up to 7 days; examine daily.

INTERPRETATION OF THE TEST
Positive Test - Blue-green pigment diffusing into the agar surrounding growth

Negative Test - No blue-green pigment produced

QUALITY CONTROL
All lot numbers of Pseudomonas P Agar have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Pseudomonas aeruginosa ATCC®27853	Ambient, up to 48 h @ 33-37°C	Growth, blue-green pigment
Stenotrophomonas maltophilia ATCC®13637	Ambient, up to 48 h @ 33-37°C	Growth, no pigment

LIMITATIONS
1. Occasional strains of *P. aeruginosa* may fail to produce pyocyanin.[3]
2. Some strains of *P. aeruginosa* may produce brown-black pyomelanin or red pyorubin which may mask pyocyanin production on Pseudomonas P Agar, resulting in a variety of hues from blue to red.[2]
3. Pyocyanin is soluble in chloroform. If pigment production is questionable (small amount of blue-green color) confirm the reaction by extraction with chloroform. Add several drops of chloroform to the growth and observe for a blue-green color.[2]

BIBLIOGRAPHY

1. King, E.O., M.K. Ward, and D.E. Raney. 1954. J. Lab. Clin. Med. 44:301-307.
2. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.
3. Gaby, W.L. and E. Free. 1953. J. Bacteriol. 65:746.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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