

PHENYLALANINE AGAR

INTENDED USE

Remel Phenylalanine Agar is a solid medium recommended for use in qualitative procedures to aid in the differentiation of enteric gram-negative bacilli based on the deamination of phenylalanine.

SUMMARY AND EXPLANATION

In 1950, Henriksen reported *Proteus* species were able to convert phenylalanine to phenylpyruvic acid.¹ In further testing, Buttiaux et al. described a method for detection of phenylpyruvic acid, formed as a result of phenylalanine deamination.² Other investigators demonstrated the usefulness of this reaction in the identification of other enteric gram-negative bacilli (e.g., *Morganella* and *Providencia*).^{3,4} Bynae incorporated phenylalanine into an agar used to grow the organism.^{5,6} Ewing et al. modified the formulation of Bynae by omitting proteose peptone.⁷ Phenylalanine Agar is recommended by the Association of Official Analytical Chemists (AOAC).⁸

PRINCIPLE

Yeast extract supplies B-complex vitamins and other nutrients to support the growth of bacteria. Sodium chloride is a source of essential electrolytes and maintains osmotic equilibrium. The substrate in this medium is phenylalanine which undergoes oxidative deamination, catalyzed by an amino acid oxidase enzyme to yield the alpha keto acid, phenylpyruvic acid. Ferric Chloride (10%) added to Phenylalanine Agar after incubation serves as a chelating agent, which combines with phenylpyruvic acid to form a green color.

REAGENTS (CLASSICAL FORMULA)*

Sodium Chloride.....	5.0 g	Disodium Phosphate	1.0 g
Yeast Extract.....	3.0 g	Agar.....	12.0 g
DL-Phenylalanine.....	2.0 g	Demineralized Water.....	1000.0 ml

pH 7.3 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE

1. Using a heavy inoculum from a pure, 18-24 hour culture, inoculate the surface of the Phenylalanine Agar slant.
2. Incubate the tube aerobically with cap loosened for 18-24 hours at 33-37°C. (If the medium is heavily inoculated, 4 hours incubation may be sufficient.⁷)
3. Add 4-5 drops of 10% Ferric Chloride (REF R21218) to the slant and gently rotate the tube.
4. Examine for a green color development on the slant and in the fluid.

INTERPRETATION OF THE TEST

Positive Test - Green color development within 1 to 5 minutes

Negative Test - No color development, medium remains yellow

QUALITY CONTROL

All lot numbers of Phenylalanine 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

Proteus vulgaris ATCC® 6380
Escherichia coli ATCC® 25922

INCUBATION

Aerobic, 18-24 h @ 33-37°C
Aerobic, 18-24 h @ 33-37°C

RESULTS

Positive
Negative

LIMITATIONS

1. Because the green color of a positive test fades quickly, the test must be interpreted within the first 5 minutes after addition of 10% Ferric Chloride.⁷

BIBLIOGRAPHY

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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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