



Phenylalanine Agar w/ 1.2% Agar

M281F

Intended Use:

Recommended for identification of *Yersinia* species in accordance with FDA BAM

Composition**

Ingredients	Gms / Litre
Yeast extract	3.000
DL-Phenylalanine	2.000
Disodium hydrogen phosphate	1.000
Sodium chloride	5.000
Agar	12.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 23.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 10 minutes. Allow the tubed medium to cool in a slanting position.

Principle And Interpretation

The ability of the bacteria to convert phenylalanine to phenylpyruvic acid is an important reaction in the differentiation of *Enterobacteriaceae* (3,7). Based on this criterion, Buttiaux developed Phenylalanine Agar for differentiation of *Proteus* and *Providencia* group from other members of *Enterobacteriaceae* (1,6). Phenylalanine Agar w/1.2% Agar is used for the differentiation of *Yersinia* species in accordance with FDA BAM, 1998 (2). *Yersinia* species are negative to phenyl alanine deaminase. Yeast extract in the medium supports the growth of the organisms. Sodium chloride maintains osmotic equilibrium. The phenylalanine serves as the substrate for enzymes, which are able to deaminate it to form phenylpyruvic acid.

Type of specimen

Food samples

Specimen Collection and Handling:

Inoculate the slant surface with plenty of inoculum and incubate it for 12-16 hours. After incubation, add 2-3 drops of 10% ferric chloride solution so that the solution floods all over the growth. Appearance of a light to deep green color indicates positive reaction and no color change indicates negative reaction. In a positive reaction, any phenylpyruvic acid present will react with the ferric salt in the reagent to give a green color. Interpret the results within 5 minutes upon addition of reagent as the green colour fades quickly (7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further biochemical and serological tests must be carried out for further identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel

Colour and Clarity of prepared medium

Light amber coloured slightly opalescent gel forms in tubes as slants **Reaction**

Reaction of 2.4% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 12-16 hours

Organism	Inoculum (CFU)	Growth	Phenylalanine deaminase
<i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50-100	luxuriant	negative reaction
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	negative reaction
<i>Proteus mirabilis</i> ATCC 25933	50-100	luxuriant	positive reaction, green colouration after addition of 10% ferric chloride
<i>Proteus vulgaris</i> ATCC 13315	50-100	luxuriant	positive reaction, green colouration after addition of 10% ferric chloride
<i>Providencia alcalifaciens</i> ATCC 9886	50-100	luxuriant	positive reaction, green colouration after addition of 10% ferric chloride

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

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3. Henrikson, S. D. 1950. J. Bacteriol, 60.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. MacFaddin, J. F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria vol. 1. Baltimore: Williams and Wilkins.
7. Singer, J. and Volcani, B. E. 1955. J. Bacteriol, 69.

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