Phenolphthalein Phosphate Agar

M652

Phenolphthalein Phosphate Agar is recommended for identification of phosphatase positive *Staphylococcus aureus* .

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Beef extract	3.000
Sodium chloride	5.000
Sodium phenolphthalein phosphate	0.012
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 28.01 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Mix well and dispense as desired.

Principle And Interpretation

Bacteria in the genus *Staphylococcus* are pathogens of man and other mammals. Traditionally they were divided into two groups on the basis of their ability to clot blood plasma (the coagulase reaction). The coagulase-positive *staphylococci* constitute the most pathogenic species *Staphylococcus aureus* . The presence of *staphylococci* in a lesion might first be suspected after examination of a direct gram stain. However, small numbers of bacteria in blood preclude microscopic examination and require culturing first (1). Phosphatase has been implicated as a virulence factor for *S. aureus* . The organisms produce both an acid and alkaline phosphates, the latter being repressed in the presence of inorganic phosphate in the medium.

Phenolphthalein Phosphate Agar is used for the identification of phosphatase-positive colonies of *S. aureus* , which is a coagulase-positive pathogenic strain (2).

Peptic digest of animal tissue and beef extract supply the nitrogenous compounds, growth factors and trace ingredients essential for the growth of *Staphylococcus aureus* . Sodium phenolphthalein phosphate serves as a substrate for the phosphatase enzyme. Sodium chloride maintains osmotic equilibrium. Phosphatase production is determined by the liberation of phenolphthalein, which is indicated by the change in colour of the medium (3). When alkali is added to this medium, the liberated phenolphthalein gives a bright pink-red colouration (4). Alternatively phosphatase production can be determined by following technique.

Technique :Grow *staphylococci* overnight at 37°C on the medium. Invert the plate and pour few drops of ammonia solution into the lid, read a positive culture whose colonies turn bright pink within a few minutes. The colour soon fades.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Yellow coloured clear to slightly opalescent gel forms in tubes as slants

Reaction
Reaction of 2.8% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response

M652: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Phosphatase
Escherichia coli ATCC 25922	50-100	luxuriant	negative, no bright pink colour on addition of alkali
Staphylococcus aureus ATCC 25923	50-100	luxuriant	positive, bright pink colour on addition of alkali
Staphylococcus epidermidis ATCC 12228	50-100	luxuriant	positive, bright pink colour on addition of alkali

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

1. Easmon C. S. F., Adlam C., 1983, Staphylococci and staphylococcal infections. Vol. 1 and 2, Academic Press, London,
2. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
3. Lewis B., 1961, J. Med. Lab. Technol., 18 : 112.
4. Barber M. and Kuper S. W. A., 1951, J. Pathol. Bacteriol., 63:65.

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