



TPEY Agar Base

M402

TPEY Agar Base with addition of supplement, it is recommended for selective isolation and enumeration of Staphylococci from foods.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Yeast extract	5.000
D-Mannitol	5.000
Sodium chloride	20.000
Lithium chloride	2.000
Agar	18.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 60 grams in 890 ml of distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 118°C (12 lbs pressure) for 15 minutes. Cool to 50°C. Aseptically add 10 ml of sterile 1% Potassium Tellurite (FD052), 100 ml Egg Yolk Emulsion (FD045) and polymyxin B to a final concentration of 4 mg/l. Mix well and pour into sterile Petri plates.

Warning : Lithium chloride is harmful. Avoid bodily contact and inhalation of vapours. On contact with skin, wash with plenty of water immediately.

Principle And Interpretation

Tellurite Polymyxin Egg Yolk Agar was formulated by Crisley et al for the detection and enumeration of coagulase-positive staphylococci in food materials (1, 2). It is also used for the recovery of coagulase-positive staphylococci from foods, air, dust and soil. The base must be supplemented with potassium tellurite solution and enriched with an egg yolk emulsion prior to its use for the isolation of staphylococci. Coagulase-negative staphylococci and other organisms are greatly suppressed on this medium. Casein enzymic hydrolysate, yeast extract and mannitol serve as nitrogenous and energy source for coagulase-positive staphylococci which adopt fermentative pathway for the utilization of carbohydrate. Lithium chloride, potassium tellurite and polymyxin B restrict the growth of wide range of bacteria including some coagulase-negative staphylococci. The coagulase-positive staphylococci are differentiated by their formation of jet black or dark grey colonies with a zone of precipitated egg yolk around the colonies or a clear zone around the colonies and precipitation below the colonies. Coagulase-negative organisms may produce small black pinpoint colonies without egg yolk precipitation or clearing around the colonies.

Mannitol-positive and/or tellurite-positive staphylococci are coagulase-negative. Definitive identification of *S. aureus*, therefore, should be based primarily on the coagulase reaction, with mannitol fermentation and tellurite reduction being used only for confirmation (3). The prepared medium becomes less inhibitory to coagulase-negative strains of staphylococci if stored for longer than one week. Graves and Frazier (4) showed that *Bacillus* species able to grow on TPEY Agar produce an antibiotic that inhibits growth of staphylococci.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.8% Agar gel.

Colour and Clarity of prepared medium

Basal medium: Light amber coloured clear to slightly opalescent gel. On addition of egg yolk emulsion and potassium tellurite, yellow coloured opaque gel forms in Petri plates.

Reaction

Reaction of 6.0% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

M402: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours with added sterile 1% Potassium Tellurite (FD052) and Egg Yolk Emulsion (FD045) and polymyxin B.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Lecithinase
<i>Bacillus subtilis</i> ATCC 6633	50-100	poor-fair	10-20%	brown	negative
<i>Escherichia coli</i> ATCC 25922	>=10 ³	inhibited	0%	-	-
<i>Proteus mirabilis</i> ATCC 25933	50-100	poor-fair	10-20%	brown	negative
<i>Staphylococcus aureus</i> ATCC 25923	50-100	good-luxuriant	>=50%	black	positive, halo or clear zone around the colony
<i>Staphylococcus epidermidis</i> ATCC 12228	50-100	poor-fair	10-20%	black	negative
<i>Streptococcus pyogenes</i> ATCC 19615	>=10 ³	inhibited	0%	-	-

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. Crisley F. D., Angelotti R. and Foter M. J., 1964, Public Health Rep. 79: 369.
2. Crisley F. D., Peeler J. F. and Angelotti R., 1965, Appl. Microbiol., 13: 140.
3. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams & Wilkins, Baltimore, Md.
4. Graves R. R. and Frazier W. C., 1963, Appl. Microbiol., 11:513.

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