K.R.A.N.E.P. Agar Base

M583

K.R.A.N.E.P. Agar Base is used for selective enumeration of total Staphylococci from food stuffs.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Sodium chloride	5.000
Yeast extract	1.500
Beef extract	1.500
Potassium thiocyanate	25.500
Sodium pyruvate	8.200
Mannitol	5.100
Lithium chloride	5.100
Sodium azide	0.050
Cycloheximide	0.041
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 71.99 grams in 900 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add sterile 100 ml of Egg Yolk Emulsion (FD045). Mix well and pour into sterile Petri plates.

Warning: Cycloheximide is very toxic and lithium chloride is harmful. Avoid skin contact or aerosol formation and inhalation. Sodium azide has a tendency to form explosive metal azides with plumbing materials, use enough water to flush off the disposables .

Principle And Interpretation

K.R.A.N.E.P. Agar is a selective medium used for the enumeration of *Staphylococcus aureus* in foods, which was first described, by Sinell and Baumgart (1). The name K.R.A.N.E.P. Agar comes from the initial letters of its main diagnostic, selective and stimulatory agents like Kalium-Rhodanid-Actidione-Natriumazid-Eigelb-Pyruvate.

The medium is selective for the detection of Staphylococci due to the presence of potassium thiocyanate and mannitol (2). The selectivity is further enhanced by the addition of sodium azide and cycloheximide (3). Sodium pyruvate and egg yolk emulsion added to the medium serve as growth enhancer and diagnostic agent respectively (4, 5). K.R.A.N.E.P. Agar is recommended for the selective isolation of coagulase negative Staphylococci from meat products (6, 7) and therefore this medium is used to enumerate the total staphylococcal count i.e. coagulase positive a and coagulase negative Staphylococci, from food products.

Peptic digest of animal tissue, yeast extract and beef extract in the medium supplies essential growth nutrients including B complex nutrients. Cycloheximide inhibits most of the yeasts and moulds. Inclusion of sodium azide helps to inhibit the accompanying aerobic organisms like *Bacillus* species, which interfere with the cultivation of Staphylococci (8). Due to the presence of inhibitory agents, various gram-negative bacteria as well as gram-positive bacteria fail to grow on this medium (4, 9).

Inoculation can be done by spread plate technique using 0.1 ml inocula on Petri plates or 0.05 ml each from different decimal dilution steps in drop plate technique. After incubation of 48 hours, well-grown golden yellow colonies with a precipitation zone of egg yolk in the medium, which remains opaque, are considered as *S.aureus* . Confirmatory tests for coagulase production are required. Colonies typical for *S.aureus* but without an egg yolk reaction should also be tested for coagulase and if positive their identity should be confirmed by further tests (10-12).

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Basal medium : Light yellow coloured clear to slightly opalescent gel. After addition of Egg Yolk Emulsion : Yellow coloured opaque gel forms in Petri plates.

Reaction
Reaction of 7.2% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
M583: Cultural characteristics observed with added sterile Egg Yolk Emulsion(FD045) after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colony characteristics	Lecithinase activity
Staphylococcus aureus ATCC 25923	50-100	luxuriant	>=50%	golden shiny	positive, opaque zone around the colony
Staphylococcus epidermidis ATCC 12228	50-100	luxuriant	>=50%	white shiny	negative
Escherichia coli ATCC 25922	>=10^3	inhibited	0%		
Candida albicans ATCC 10231	>=10^3	inhibited	0%		
Bacillus subtilis ATCC 6633	>=10^3	inhibited	0%		

Storage and Shelf Life

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

Reference

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Revision : 2 / 2015