Kohn Two Tube Medium No. 2

M802

Kohn Two Tube Medium No. 2 is used for the identification of members of *Enterobacteriaceae* on the basis of sucrose and salicin fermentation, motility, H2S and indole production.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Casein enzymic hydrolysate	10.000
Sucrose	10.000
Salicin	10.000
Sodium chloride	5.000
Sodium thiosulphate	0.016
Disodium hydrogen orthophosphate	0.090
Bromothymol blue	0.020
Agar	3.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 48.13 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes. Sterilize by autoclaving at 115°C(10 lbs pressure) for 15 minutes. Cool the tubed medium in an upright position.

Principle And Interpretation

Russell (1) first introduced Double Sugar Medium, a differentiating medium for *Enterobacteriaceae* . Kohn (2) later developed a technique employing two tubes of composite media for study of culture reactions, for the identification of *Enterobacteriaceae* . Gillies (3) further made minor modifications in Kohns media. Kohn Two Tube Medium No.2 is used to study carbohydrate fermentation (Sucrose and Salicin) along with motility, hydrogen sulfide production and indole production.

Using a straight wire, inoculate with a single stab to about one-third of the depth of the Kohn Two Tube Medium No. 2. Suspend the two test papers (lead acetate and Kovacs) above the medium by bending and trapping them between the cotton wool plug and the side of the test tube. Incubate at 37°C for 18-24 hours and examine for motility, H2S production, sugar fermentation and indole production. Motility is seen as diffused growth spreading from the line of inoculation. The blackening of the lead acetate paper strip indicates H2S production. Fermentation of sucrose or salicin or both is indicated by the colour change to yellow with bromothymol blue being the pH indicator. Indole formation is indicated by the change in colour of the Kovacs reagent paper to pinkish red.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Semisolid, comparable with 0.3% Agar gel.

Colour and Clarity of prepared medium
Green coloured, clear to slightly opalescent gel forms in tubes as butts

Reaction
Reaction of 4.81% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
M802: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours .

Organism	Inoculum (CFU)	Motility	Fermentation w/ Sucrose/ Salicin	H2S(with lead acetate strip)	Indole
Proteus vulgaris ATCC 13315	50-100	positive, growth away from stabline causing turbidity	acid & gas production or negative reaction	variable reaction	variable reaction
Salmonella Typhimurium ATCC 14028	50-100	positive, growth away from stabline causing turbidity	negative reaction	variable reaction	negative reaction
Salmonella Typhi ATCC 6539	50-100	positive, growth away from stabline causing turbidity	negative reaction	positive, blackening of the lower portion of the strip	negative reaction
Shigella flexneri ATCC 12022	50-100	negative, growth along the stabline, surrounding medium remains clear	negative reaction	negative,no blackening	variable
Shigella sonnei ATCC 25931	50-100	negative, growth along the stabline, surrounding medium remains clear	negative reaction	negative,no blackening	negative reaction
Shigella schmitzi	50-100	negative, growth along the stabline, surrounding medium remains clear	negative reaction	negative,no blackening	positive reaction,pink colour at the lower portion of the strip

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

1.Russell F. F., 1911, J. Med. Res., 25:217.
2.Kohn J., 1954, J. Path. Bacteriol., 67(1): 286.
3.Gillies R. R., 1956, J. Clin. Pathol., 9(4):368.

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