

Technical Information

Kohn Two Tube Medium No. 2

Product Code: DM 1802

Application: - 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

Principle & Interpretation

Russell ⁽¹⁾ first introduced Double Sugar Medium for differentiating member of family *Enterobacteriaceae*. Kohn ⁽²⁾ later developed a technique using two tubes of composite media to study culture reactions, for the identification of member 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, 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.

Methodology

Suspend 48.12 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Dispense in tubes. Sterilize by autoclaving at 115°C for 15 minutes. Cool the tubed medium in an upright position.

Quality Control

Physical 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 Range 7.20-7.60

Cultural Response/Characteristics

DM 1802: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Motility	Fermentation w/ Sucrose/ Salicin	H ₂ S(with lead acetate strip)	Indole
<i>Proteus vulgaris</i> ATCC 13315	50-100	positive, growth away from stabline causing turbidity	acid & gas production or negative reaction	Variable reaction	Variable reaction
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	positive, growth away from stabline causing turbidity	negative reaction	Variable reaction	negative reaction
<i>Salmonella Typhi</i> ATCC 6539	50-100	positive, growth away from stabline causing turbidity	negative reaction	positive Blackening of the lower portion of the strip	negative reaction
<i>Shigella flexneri</i> ATCC 12022	50-100	negative, growth along the stabline, surrounding medium remains clear	negative reaction	negative, no blackening	Variable
<i>Shigella sonnei</i> ATCC 25931	50-100	negative, growth along the stabline, surrounding medium remains clear	negative reaction	negative, no blackening	negative reaction
<i>Shigella schmitzi</i>	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

Dried media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8⁰ in sealable plastic bags for 2-5 days.

Further Reading

1. Russell F. F., 1911, J. Med. Res., 25:217.
2. Kohn J., 954, J. Path. Bacteriol., 67(1): 286.
3. Gillies R. R., 1956, J. Clin. Pathol., 9(4):368.



Dehydrated Culture Media
Bases / Media Supplements

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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