



## Kohn Two Tube Medium No. 1 Base

M142

Kohn Two Tube Medium No. 1 Base is used for the identification of *Enterobacteriaceae* on the basis of dextrose and mannitol fermentation and urease production.

### Composition\*\*

Ingredients	Gms / Litre
Peptic digest of animal tissue	15.000
Beef extract	2.000
Yeast extract	2.000
Dextrose	1.000
Mannitol	10.000
Phenol red	0.050
Agar	16.000
Final pH ( at 25°C)	7.2±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 46.05 grams in 975 ml distilled water . Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 115°C (10 lbs pressure) for 15 minutes.Cool to 60°C and aseptically add 25 ml of sterile 40% (w/v) Urea solution (FD048). Mix well and make slants with a generous butt.

### 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.1 Base is used to study dextrose and mannitol fermentation along with urease production.

Inoculate pure culture of organisms with a straight wire by stabbing the butt and smearing the surface of the slope of Kohn Two Tube Medium No.1 Base. Incubate at 37°C for 18 hours. Phenol red is the pH indicator . Organisms capable of fermenting only dextrose show a yellow butt with or without gas formation and the slant remains unchanged (red). A yellow slant indicates the fermentation of mannitol. A positive urease reaction is shown by a deep cerise (light red) colour of whole medium.

### Quality Control

#### Appearance

Light yellow to light pink homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.6% Agar gel.

#### Colour and Clarity of prepared medium

Pink coloured, clear to slightly opalescent gel forms in tubes as slants with a generous butt

#### Reaction

Reaction of 4.6% w/v aqueous solution at 25°C. pH : 7.2±0.2

#### pH

7.00-7.40

#### Cultural Response

M142: Cultural characteristics observed with added sterile 40% w/v Urea Solution (FD048) after an incubation at 35-37°C for 18-24 hours

Organism	Inoculum (CFU)	Fermentation of Dextrose	Fermentation of Mannitol	Urease production
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<i>Proteus vulgaris</i> ATCC 13315	50-100	apparent negative reaction, urease activity masks fermentation reaction.	apparent negative reaction, urease activity masks fermentation reaction.	positive reaction, cerise colour
<i>Salmonella Typhi</i> ATCC 6539	50-100	acid production, yellow colour	acid production, yellow colour	negative reaction, no change
<i>Salmonella Enteritidis</i> ATCC 13076	50-100	acid & gas production, yellow colour	acid production, yellow colour	negative reaction, no change
<i>Shigella flexneri</i> ATCC 12022	50-100	acid production, yellow colour	acid production, yellow colour	negative reaction, no change
<i>Shigella sonnei</i> ATCC 25931	50-100	acid production, yellow colour	acid production, yellow colour	negative reaction, no change

### Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8°C in tightly closed container. 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.

Revision : 1 / 2011



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