

## Technical Information

### Kohn Two Tube Medium No. 1

#### Product Code: DM 1142

**Application:** Kohn Two Tube Medium No. 1 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

#### Principle & Interpretation

Russell <sup>(1)</sup> was the to first introduce Double Sugar Medium, for differentiating member of *Enterobacteriaceae family*. Kohn <sup>(2)</sup> later developed a technique using two tubes of composite media to study of culture reactions, and for the identification of *Enterobacteriaceae*. Gillies <sup>(3)</sup> further made minor modifications in Kohns media. Kohn Two Tube Medium No.1 Base is used to study fermentation of dextrose and mannitol 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.

#### Methodology

Suspend 46.05 grams of powder media in 975 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 115°C for 15 minutes. Cool to 60°C and aseptically add 25 ml of sterile 40% (w/v) Urea solution (MS 2048). Mix well and make slants with a

#### Quality Control

##### Physical 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 Range:- 7.00-7.40

### Cultural Response/Characteristics

DM 1142: Cultural characteristics observed with added sterile 40% w/v Urea Solution (MS2048) after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Fermentation of Dextrose	Fermentation of Mannitol	Urease Production
<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

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

**Prepared Media:** 2-8<sup>o</sup> in sealable plastic bags for 2-5 days.

## Further Reading

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.

## Disclaimer :

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