



Kohn Two Tube Medium No.1 Base

M142

Intended Use:

Recommended for identification of *Enterobacteriaceae* on the basis of dextrose and mannitol fermentation and urease production.

Composition**

Ingredients	g / L
Peptone	15.000
HM peptone B #	2.000
Yeast extract	2.000
Dextrose(Glucose)	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

Equivalent to Beef extract

Directions

Suspend 46.05 grams in 975 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 115°C (10 lbs pressure) for 15 minutes. Cool to 45-50°C and aseptically add 25 ml of sterile 40% (w/v) U40 Supplement (5 ml per vial)(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.

Type of specimen

Clinical isolates

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Slight colour variation may be observed depending upon strains.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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

Cultural characteristics observed with added sterile 40% w/v U40 Supplement (5 ml per vial) (FD048) after an incubation at 35-37°C for 18-24 hours

Organism	Fermentation of Dextrose	Fermentation of Mannitol	Urease production
## <i>Proteus hauseri</i> ATCC 13315	apparent negative reaction, urease activity masks fermentation reaction.	apparent negative reaction, urease activity masks fermentation reaction.	positive reaction, cerise colour
<i>Salmonella</i> Typhi ATCC 6539	acid production, yellow colour	acid production, yellow colour	negative reaction, no change
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	acid & gas production, yellow colour	acid production, yellow colour	negative reaction, no change
<i>Shigella flexneri</i> ATCC 12022 (00126*)	acid production, yellow colour	acid production, yellow colour	negative reaction, no change
<i>Shigella sonnei</i> ATCC 25931	acid production, yellow colour	acid production, yellow colour	negative reaction, no change

Key : (*) Corresponding WDCM numbers. ## Formerly known as *Proteus vulgaris*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

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.
4. Isenberg, H. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

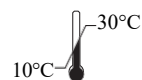
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