Kohn Two Tube HiVeg™ Medium No.2

MV802

Intended Use:

Recommended for identification of *Enterobacteriaceae* on the basis of sucrose and salicin fermentation, motility, hydrogen sulphide and indole production.

Composition**

Ingredients	Gms / Litre
HiVeg™ peptone	10.000
HiVeg™ hydrolysate	10.000
Saccharose (Sucrose)	10.000
Salicin	10.000
Sodium chloride	5.000
Sodium thiosulphate	0.016
Disodium hydrogen phosphate	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 purified / 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 HiVeg™ Medium No.2 is prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks. Kohn Two Tube Medium No.2 is used to study carbohydrate fermentation (Sucrose and Salicin) along with motility, hydrogen sulfide production and indole production.

Type of specimen

Samples

Specimen Collection and Handling

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 Kovac's) 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, H_2S 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 H_2S 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. After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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. Well isolated colonies must be used.

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

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

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 (00031*)	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 (00126*)	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

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°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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