B.C.G.-Dextrose HiVeg® Agar (Snyder test HiVeg® Agar)

MV106

Intended Use

Recommended for the estimation of Lactobacilli, as an indication of caries activity.

Composition**

Ingredients	g / L
HiVeg® peptone	20.000
Dextrose (Glucose)	20.000
Sodium chloride	5.000
Bromocresol green	0.020
Agar	20.000
Final pH (at 25°C)	4.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 65.02 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense in 10 ml amounts into test tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in an upright position. **DO NOT OVERHEAT** the medium.

Principle And Interpretation

B. C. G dextrose HiVeg® Agar (Snyder Test HiVeg® Agar) is prepared by replacing the animal based peptone with the vegetable based HiVeg® peptone, making the medium BSE/TSE risks free. Dental caries results from microbial acid and plaque formation. Plaque sets the stage for caries because it collects the acid forming bacteria on the tooth surface, supplies an anaerobic environment for fermentation, traps the acids and excludes the protective saliva. Caries lesions are basically the outcome of chemical attack on the enamel and dentin. Demineralization of the tooth alternates with periods of re-mineralization. If demineralization exceeds re-mineralization, a subsurface carious lesion becomes a clinical cavity with extension of the decay into the dentine (1). For determining the rate and amount of acid produced by microorganisms in saliva, Snyder (2,3) described a colorimetric method. The procedure makes use of an agar medium that is known as Snyder Test Agar. Later on Alban (4) modified the procedure and reported it to be more accurate than the original procedure. B. C. G. Dextrose HiVeg® Agar is the modification of B. C. G. Dextrose Agar and serves the same purpose.

This is a differential medium based on the rate of acid production from dextrose, by oral acidogenic microorganisms from buccal cavity and is evidenced by a change in colour of the indicator - bromo cresol green from blue-green to yellow. HiVeg® peptone provides carbon, nitrogen, long chain amino acids, vitamins and minerals. Dextrose is the carbohydrate source and bromo cresol green is the pH indicator.

Snyder

Test Procedure:

Collect specimens of saliva before breakfast, before brushing the teeth or just before lunch or dinner. Collect specimen of saliva in a sterile tube or bottle after patient chews paraffin for 3 minutes. Shake the specimen thoroughly and transfer 0.2 ml of this to a sterile Snyder Test Agar tube melted and cooled to 45°C. Mix the inoculum by rotating the inoculated tubes and incubate at 37°C for 72 hours in an upright position. The rate of acid production is graded as, marked for 24 hours, moderate and slight if colour changes within 48 and 72 hours respectively (5).

Incubation hours	Colour	Caries activity
24	yellow	marked
48	greenish yellow	moderate
72	yellowish green	slight

Alban Modified Test Procedure:

Collect the saliva specimen (unstimulated) to just cover the medium in the tube. When specimen collection is difficult, dip a sterile cotton swab into the saliva under the tongue or rub on tooth surfaces and place the swab just below the surface of the medium. Incubate the tubes at 35°C along with uninoculated control. Examine tubes daily for 4 days and compare the colour change with the control tube.

Record the results as:

No colour change as negative Colour : -

beginning to change to yellow Half : +

medium yellow : ++

Three fourths of medium yellow Total : +++

medium yellow : ++++

The daily readings indicate the rapidity and amount of acid production. To establish a reference point at least two specimens collected within 2-4 days must be obtained.

Type of specimen

Clinical samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Some organisms may show poor growth due to variable nutritional requirements.

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 greenish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium

Emerald green coloured, clear to slightly opalescent gel forms in tub

pH 4.60-5.00

Reaction

Reaction of 6.5% w/v aqueous solution at 25°C. pH : 4.8±0.2

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-72 hours.

Organism	Inoculum (CFU)	Growth	Acid production
Lactobacillus acidophilus ATCC 314	50-100	good-luxuriant	positive reaction, yellow colour
Lactobacillus casei ATCC 9595	50-100	good-luxuriant	positive reaction, yellow colour

Lactobacillus fermentum ATCC 9338	50-100	good-luxuriant	positive reaction, yellow colour
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	none-poor	negative reaction,no colour change

Key : *Corresponding WDCM numbers.

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

1.Lewis and Ismail, 1995, Can. Med. Assoc. J., 152:836.
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4.Alban, 1970, J. Dent. Res., 49:641.
5.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams & Wilkins, Baltimore, Md.
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7.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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