HS Medium

M245

Intended Use:

Recommended for cultivation of aerobic as well as anaerobic bacteria and sterility testing.

Composition**

Ingredients	Gms / Litre
Tryptone	15.000
Yeast extract	5.000
Sodium dithionite (Sodium hydrosulfite)	0.500
Sodium chloride	2.500
Dextrose (Glucose)	5.500
Resazurin	0.001
Agar	1.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.5 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
Note: If more than the upper one-third of the medium has acquired a pink colour, the medium may be restored once by heating in a water bath or in free flowing steam until the pink colour disappears.

Principle And Interpretation

Anaerobic bacteria are widespread in soil, marshes, lake and river sediments, oceans, sewage, foods and animals. In humans, anaerobic bacteria normally are prevalent in the oral cavity around the teeth, in the gastrointestinal tract, especially in the colon. Most of these anaerobic habitats have both a low oxygen tension and reduced Eh, resulting from the metabolic activity of microorganisms that consume oxygen through respiration. If the oxygen is not replaced, anaerobic conditions are maintained in the environment. The media used for recovering anaerobes from specimen should include non-selective, selective and enrichment types.
HS Medium was described by Bonnel and Raby for use in sterility testing (1). It is similar to Fluid Thioglycollate Medium (M009) where sodium hydrosulphite is substituted for sodium thioglycollate, in the latter, to obtain oxidized and reduced conditions which are appropriate for the growth of aerobes as well as anaerobes (1,2). HS medium can be used for the sterility testing of biological and pharmaceutical products. Bonnel and Raby used HS Medium for control tests on blood products and for isolation of *Corynebacterium*, Streptococci, Staphylococci, enteric bacilli, *Neisseria*, Clostridia etc .
Tryptone and yeast extract in the medium supply essential nutrients such as amino acids, carbon, sulphur and minerals. Sodium hydrosulphite helps to create anaerobic atmosphere, as it is an oxygen scavenger. Dextrose is the fermentable carbohydrate and resazurin is the redox indicator dye. Sodium chloride helps to maintain the osmotic equilibrium of the medium.

Type of specimen

Pharmaceutical samples for sterility testing

Specimen Collection and Handling:

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (1,2,7)
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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further biochemical and serological tests must be carried out for further identification.

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

Colour and Clarity of prepared medium

Light straw coloured, clear to slightly opalescent solution with upper 10% or less medium having pinkish tinge on standing.

Reaction

Reaction of 2.95% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

Cultural characteristics observed after an incubation (i)bacteria at 35-37°C (ii) *Clostridium* species anaerobically for 18-48 hours.

Organism	Inoculum (CFU)	Growth
Clostridium perfringens ATCC 12924	50-100	good-luxuriant
Corynebacterium diphtheriae ATCC 11913	50-100	good-luxuriant
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	good-luxuriant
Staphylococcus aureus subsp. *aureus* ATCC 25923	50-100	good-luxuriant
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant

Key : (*) Corresponding WDCM numbers. (#) Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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 (1,2).

Reference

1. Bonnel and Raby, 1958, Proc. 7th Cong. Int. Soc. Blood Transfusion,317, Rome.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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.
4. WHO, 1960, Technical Report Series No. 200, WHO, Geneva.P

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