Clausen Medium

M552

Intended Use

Recommended for sterility testing as per Nordic Pharmacopoeia Board.

Composition**

Ingredients	Gms / Litre
Tryptone	15.000
Soya peptone	3.000
Yeast extract	6.000
Dextrose (Glucose)	6.000
Sodium chloride	2.500
Dipotassium hydrogen phosphate	2.000
Sodium citrate	1.000
L-Cystine	0.500
L-Asparagine	1.250
Sodium dithionite	0.400
Sodium thioglycollate	0.500
Lecithin	0.300
Magnesium sulphate	0.400
Calcium chloride	0.004
Cobalt sulphate	0.001
Cupric sulphate	0.001
Ferrous sulphate	0.001
Zinc sulphate	0.001
Manganese chloride	0.002
Resazurin	0.001
Agar	0.750
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40 grams in 1000 ml purified / distilled water containing 3 grams polysorbate 80 and 5 grams glycerol. Heat to boiling to dissolve the medium completely. Dispense into tubes or flasks as desired and sterilize by autoclaving at 118°C for 15 minutes. Place in cool dark place till use. DO NOT RESTERILIZE the medium.

Note: If more than 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

Clausen Medium was developed by Clausen (1). This medium is also called as HS-T (Dithionite Thioglycollate) Medium and is recommended for sterility testing by the Nordic Pharmacopoeia Board. Random sample selection is recognized by the Board and they refer to the process as microbial-contamination test. The Standard microbial contamination test is developed to establish the number of non-sterile units, if any in batch, is below a specific level. Random sampling in sufficient quantity of the bulk should be examined.

In the microbial contamination test for detecting the non-sterile units, two methods can be used viz. Membrane filter method and Dilution method. The test must be performed with all precautions taken to prevent laboratory contamination.

This medium is very nutritious consisting of Tryptone, Soya peptone, yeast extract and dextrose. L-cystine and sodium thioglycollate act as reducing agents, and the essential metals help for isolating anaerobic spore-formers. Polysorbate 80 and lecithin are added in this medium to overcome the effects of cationic agents, which can exert bacteriostatic effect in vitro. This medium is clear in appearance and yellow coloured. Under aerobic conditions it turns pink. Therefore at the time of use the upper one third of the medium should be pink.

The standard microbial contamination test is passed if growth is not observed in any of the tubes. Growth is examined by the appearance of turbidity in fluid or semi fluid media and by the formation of colonies on solid media.

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 (2,3,12) 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. It is intended for the examination of clear liquid or water-soluble materials.

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 portion pink on standing.

Reaction

Reaction of 4% w/v aqueous solution containing 0.3% w/v polysorbate 80 and 0.5% w/v glycerol pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth
Bacillus subtilis subsp. *spizizenii* ATCC 6633 (00003*)	50-100	luxuriant
Candida albicans ATCC 10231 (00054*)	50-100	luxuriant
Clostridium sporogenes ATCC 11437	50-100	luxuriant
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	luxuriant
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	luxuriant
Streptococcus pyogenes ATCC 19615	50-100	luxuriant

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Clausen O.G., 1973, Pharmaceutica Acta Helvetiae, 48:541.
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.

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