Clausen Medium

Intended Use
Clausen Medium is recommended by Nordic Pharmacopoeia Board for sterility testing.

Summary
Clausen Medium was developed by Clausen. This medium is also called as HS-T (Dithionite Thioglycollate) Medium and is recommended for sterility testing by the Nordic Pharmacopoeia Board. The Board identified the problems of sterility testing by selecting random samples and they refer to the process as the Microbial-Contamination Test. The standard microbial- contamination test is designed solely to establish that the number of non-sterile units, if any, in a batch is below a certain level. The tests must be performed with all precautions taken to prevent laboratory contamination occurring more than once in every 100 tests. The use of laminar air-flow cabinets is recommended. Tests are to be made by qualified and experienced staff and the efficiency of the methods used must be checked at regular intervals.
A random sample of sufficient quantity to be representative of the whole 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.

Principle
This medium is very nutritious consisting of casein enzymic hydrolysate, papaic digest of soyabean meal, yeast extract and dextrose. L-cystine and sodium thioglycollate act as reducing agents, and the essential metals help for isolating anaerobic sporeformers. Lecithin is added in this medium to overcome the effects of cationic agents, which can exert bacteriostatic effect *In vitro*.

Formula*

Ingredients	**g/L**
Casein enzymic hydrolysate	15.0
Papaic digest of soyabean meal	3.0
Yeast extract	6.0
Dextrose	6.0
Sodium chloride	2.5
Dipotassium phosphate	2.0
Sodium citrate	1.0
L-Cystine	0.5
L-Asparagine	1.25
Sodium dithionite	0.4
Sodium thioglycollate	0.5
Lecithin	0.3
Magnesium sulphate	0.4
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	0.75
Final pH (at 25°C)	7.1 ± 0.2

*Adjusted to suit performance parameters

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2ºC-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Specimen Collection and Handling

Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 40.00 g of powder in 1000 mL purified / distilled water containing 3 g polysorbate 80 and 5 g glycerol.
2. Heat to boiling to dissolve the powder completely.
3. Dispense as desired and sterilize by autoclaving at 118°C for 15 minutes as per validated cycle.
4. Place in cool dark place till use.
5. 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.

Quality Control

Dehydrated Appearance: Light yellow to yellow coloured homogeneous free flowing powder.
Prepared Appearance: Light straw coloured, very slightly opalescent solution with upper portion less than 10% medium turning pink on standing.
Growth Promotion Test: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP/IP and growth is observed after an incubation at 30°C-35°C for ≤ 3 days.
Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time, inoculating 10 - 100 cfu (at 30°C - 35°C for ≤ 3 days).

Organism (ATCC)	**Growth**
Staphylococcus aureus subsp. *aureus* (6538)	Good
Pseudomonas aeruginosa (9027)	Good
Kocuria rhizophila Strain PCI 1001 (9341)	Good
Bacteroides vulgatus (8482)	Good
Clostridium sporogenes (11437)	Good
Clostridium sporogenes (19404)	Good
Staphylococcus epidermidis strain PCI 1200 (12228)	Good

Validation and Growth Promotion: Growth promotion is carried out at an incubation of 20°C-25°C for ≤ 3 days for bacteria and ≤ 5 days for fungi as per USP/EP/JP/IP.

Organism (ATCC)	**Growth**
Candida albicans 3147 (10231)	Good
Bacillus spizizenii (6633)	Good
Aspergillus brasiliensis WLRI 034(120) (16404)	Good

Interpretation of Results

1. The standard microbial contamination test is passed if growth is not observed in any of the tubes.
2. If growth is observed, the test may be repeated with twice the number of samples. The test is then passed if no growth is observed in any of these tubes.
3. Growth is diagnosed by the appearance of turbidity in fluid or semi-fluid media, by the formation of colonies on solid media, or by microscopy of culture samples.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

The medium is yellowish in colour and almost clear. It turns pale-pink under aerobic conditions. The upper third only of the medium should be pink by the time it is to be used.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Clausen O. G., Aasgaard N. B. and Solberg O. (1973) Ann. Microbiol. (Inst. Pasteur) 124 B. 205.
2. Christensen E. A., Kristensen H. and Jensen K. M. (1969) Arch. Pharm. Chem. 76. 625.
3. Clausen O. G. (1973) `A study of the growth-promoting properties of fluid and solid microbial-contamination test media on small numbers of micro- organisms.' `Pharmaceutica Acta Helvetiae''48. 541-548.
4. Clausen O. G. (1973) `An examination of the Bactericidal and Fungicidal Effects of Cetylpyridinium Chloride, separately and in combinations embodying EDTA and Benzyl Alcohol'. Die. Pharm. Ind. 35. Nr. 12 869-874
5. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201030110100	Dehydrated Culture Media	100 g
201030110500	Dehydrated Culture Media	500 g