

Technical Information

Columbia Agar

Product Code: DM 1144H

Application: - Columbia Agar is recommended for detection of *Clostridium sporogenes* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP (Medium 15).

Composition**

Ingredients	Gms / Litre
Pancreatic digest of casein	10.000
Meat peptic digest	5.000
Heart pancreatic digest	3.000
Yeast extract	5.000
Maize starch	1.000
Sodium chloride	5.000
Agar	15.000

If necessary adjust the pH so that after sterilization it is 7.3 ± 0.2

* pH can also be measured after sterilization at 25°C

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Columbia Blood Agar Base recommended as a general-purpose nutritious medium was devised by Ellner et al from Columbia University, which was further enriched by the addition of sheep blood (1). It can also be recommended for the isolation of organisms by addition of various supplements. Columbia Agar is prepared in accordance with the microbial limit testing harmonized methodology of USP/EP/BP/JP/IP (2, 3, 4, 5, 6). This medium is used to check the presence of *Clostridium* in non-sterile products like food, dietary, nutritional supplements related products. The genus *Clostridium* belongs to the family Clostridiaceae in the class Clostridia.

The product to be examined is initially enriched in Reinforced medium for clostridia. This medium contains 0.05% Agar and cysteine, which creates anaerobic conditions, thereby allowing anaerobic organisms to grow. The enriched sample is then subcultured on Columbia Agar. Columbia Agar is used as a base for media containing blood and for selective media formulations in which different combinations of antimicrobial agents are recommended as additives.

This medium is highly nutritious as it contains pancreatic digest of casein, meat peptic digest, heart pancreatic digest and yeast extract which supports rapid and luxuriant growth of fastidious as well as non-fastidious organisms. Sodium chloride maintains osmotic balance of medium. Maize starch acts as an energy source and also neutralizes toxic metabolites if produced. It is recommended in detection of Clostridia from pharmaceutical products. Gentamicin (MS1252) inhibits a number of contaminating gram-negative organisms and Staphylococcus species.

Clostridia grows under anaerobic conditions as gram positive rods giving a catalase negative test. Further confirmation is carried out by identification tests.

Methodology

Suspend 44 grams of dehydrated media powder in 1000 ml distilled water. Mix thoroughly & heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle. Cool to 45-50°C, if required add the rehydrated contents of 1 vial of Gentamicin Selective Supplement (MS1252). Mix well before pouring into sterile Petri plates.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity

Light amber coloured clear to slightly opalescent gel forms in Petri plates.

pH Range

7.10-7.50

Growth Promotion Test

Growth Promotion was carried out in accordance with the harmonized method of USP/EP/BP/JP, and growth was observed under anaerobic conditions after an incubation at 30-35°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Casein Soybean Digest Agar (Soybean Casein Digest Agar).

Growth promoting properties

Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating ≤ 100 cfu under anaerobic conditions (at 30-35°C for ≤ 48 hours).

Cultural Response

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Incubation temperature	Incubation period
Growth Promoting						
<i>Clostridium sporogenes</i> ATCC 19404	50 -100	luxuriant	25 -100	≥ 50 %	30 -35 °C	≤ 48 hrs
<i>Clostridium sporogenes</i> ATCC 11437	50 -100	luxuriant	25 -100	≥ 50 %	30 -35 °C	≤ 48 hrs
<i>Bacteroides vulgatus</i> ATCC 8482	50 -100	luxuriant	25 -100	≥ 50 %	30 -35 °C	≤ 48 hrs
Additional Microbiological testing						
<i>Clostridium perfringens</i> ATCC 13124	50 -100	luxuriant	25 -100	≥ 50 %	30 -35 °C	≤ 48 hrs
<i>Bacteroides fragilis</i> ATCC 23745	50 -100	luxuriant	25 -100	≥ 50 %	30 -35 °C	≤ 48 hrs

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. Ellner, Stoessel, Drakeford and Vasi, 1966, Am. J. Clin. Pathol., 45:502.
2. The United States Pharmacopoeia, 2011, The United States Pharmacopoeial Convention. Rockville, MD.
3. British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia
4. European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.
5. Japanese Pharmacopoeia, 2008.
6. Indian Pharmacopoeia, 2010, Govt.of India, the Controller of Publication , New Delhi

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at CDH is true and accurate
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