



Columbia Agar

MH144

Columbia Agar is used 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

Directions

Suspend 44 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 or as per validated cycle. Cool to 45-50°C, if required add the rehydrated contents of 1 vial of Gentamicin Selective Supplement (FD252). Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Columbia Blood Agar Base used 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 used 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 recommended 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 used 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 used in detection of Clostridia from pharmaceutical products. Gentamicin (FD252) 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.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Please refer disclaimer Overleaf.

pH

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

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

Reference

- 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

Revision : 2 / 2015

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.