

PRODUCT SPECIFICATION SHEET



Columbia Agar (DM063H)

Intended Use

Columbia Agar (DM063H) is recommended for detection of *Clostridium sporogenes* from pharmaceutical products, using the microbial limit testing, in compliance with Harmonized methodology of USP/EP/IP/BP/JP.

Product Summary and Explanation

Ellner et al., from Columbia University in 1966, reported the development of a blood agar formulation, which has been designated as Columbia Agar. Columbia Blood Agar Base is a general-purpose nutritious medium, which was further enriched by the addition of sheep blood.⁽¹⁾ Columbia blood agar base media are typically supplemented with 5-10% sheep, rabbit, or horse blood for use in isolating, cultivating and determining hemolytic reactions of fastidious pathogenic microorganisms. The base achieves the more rapid and luxuriant growth obtained from casein hydrolysate media with the sharply defined hemolytic reactions, more typical colonial morphology and improved pigment production achieved with media containing infusion peptone. 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.

Principles of the Procedure

Columbia Agar contains pancreatic digest of casein, meat peptic digest, heart pancreatic digest which supports rapid and luxuriant growth of fastidious as well as non-fastidious organisms. Sodium chloride maintains osmotic balance of medium. Yeast extract serves as a supplier of the B-complex vitamins. 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 (MS094) inhibits a number of contaminating gram-negative organisms and Staphylococcus species.

Formula / Liter

Ingredients	Gms / Liter
Pancreatic digest of casein	10.00
Meat peptic digest	5.00
Heart pancreatic digest	3.00
Yeast extract	5.00
Maize starch	1.00
Sodium chloride	5.00
Agar	15.00
Final pH: 7.3 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 44 grams of the medium in one liter of purified/distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 45-50°C, if required add the rehydrated contents of 1 vial of Gentamicin Selective Supplement (MS094).
5. Mix well before pouring into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light amber coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 4.4% solution	pH 7.3 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel



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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).

Expected Cultural Response:

Sr. No.	Organisms	Results to be achieved				
		Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Incubation Temperature & Time
	Growth promoting					
1.	<i>Clostridium sporogenes</i> ATCC 19404	50 - 100	good-luxuriant	25 -100	≥ 50 %	30-35°C ≤ 48 hrs
2.	<i>Clostridium sporogenes</i> ATCC 11437	50 - 100	good-luxuriant	25 -100	≥ 50 %	30-35°C ≤ 48 hrs
3.	<i>Bacteroides vulgates</i> ATCC 8482	50 - 100	good-luxuriant	25 -100	≥ 50 %	30-35°C ≤ 48 hrs
	Additional Microbiological testing					
4.	<i>Clostridium perfringens</i> ATCC 13124	50 - 100	good-luxuriant	25 -100	≥ 50 %	30-35°C ≤ 48 hrs
5.	<i>Bacteroides fragilis</i> ATCC 23745	50 - 100	good-luxuriant	25 -100	≥ 50 %	30-35°C ≤ 48 hrs

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. 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.
2. 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.
3. Refer to appropriate references for standard test procedures.

Results

Clostridia grow under anaerobic conditions as gram positive rods giving a catalase negative test. Further confirmation is carried out by identification tests. Refer to appropriate references and standard test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.



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Packaging

Product Name : Columbia Agar

Product Code : DM063H

Available Pack sizes : 100gm/500gm

References

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. Revision : 1 / 2011.
6. Indian Pharmacopoeia, 2010, Govt. of India, the Controller of Publication, New Delhi.

Further Information

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