Antibiotic Assay Medium H (DM022I)

Intended Use

Antibiotic Assay Medium H is used as seed layer for microbiological assay of Carbenicillin, Colistimethate sodium, Colistin sulphate and Polymyxin B in accordance with Indian Pharmacopoeia.

Product Summary and Explanation

The antibiotic media are identified numerically with names assigned by Grove and Randall in *Assay Methods of Antibiotics* [1] and is in accordance to IP. The activity (potency) of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms. [2] Reduction in antimicrobial activity may reveal changes not demonstrated by chemical methods. [2] An assay is made to determine the ability of an antibiotic to kill or inhibit the growth of living microorganisms. Biological tests offer the most convenient means of performing an assay, [3] since a reduction in the antimicrobial activity of a specific antibiotic reveals changes not usually displayed by chemical methods. [4] Antibiotic assays are performed by the cylinder plate method and the turbidimetric "tube" assay. The cylinder plate method, first described by Abraham et al. [5] for the assay of penicillin, was later modified by Foster and Woodruff [6] and by Schmidt and Moyer. [7] The choice of methodology is often based on many factors, including relative ease of performance, flexibility and use of automated or semi-automated devices for both identification and susceptibility testing. [8]

Principles of the Procedure

Combination of pancreatic digest of casein and papaic digest of soya bean provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients for the growth of test organisms. Natural soya sugars enhance microbial growth. Dextrose provides the carbon source and enhances the growth of test organism. Phosphates in the medium enhance buffering action and sodium chloride maintains osmotic equilibrium. Polymyxin gives smaller zone of inhibition as it slowly diffuses into the agar [9]. Reduced agar concentration (1.2%) in this medium improves the diffusion of polymyxin into the medium. Polysorbate 80 functions synergistically with polymyxin on spheroplasts of Pseudomonas aeruginosa. Polysorbate 80 is a suitable ingredient for Polymyxin assay as it enhances the penetration of Polymyxin to the cytoplasmic membrane [10]. Freshly prepared plates should be used for antibiotic assays. Sterile seed agar which is pre-cooled to 40-45°C and spread evenly over the surface of solidified base agar is where the test organisms are inoculated. All the microbiological assay conditions must be carefully controlled. One of the important steps is to use standard culture media for good results.

Formula / Liter

Ingredients	Gms / Liter
Papaic digest of soyabean (soya peptone)	3.00
Pancreatic digest of casein (Tryptone)	17.00
Dextrose	2.50
Sodium chloride	5.00
Dipotassium hydrogen phosphate	2.50
Agar	12.00
Final pH: 7.2 ± 0.1 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.
3. Freshly prepared plates should be used for antibiotic assays.
4. All conditions in the microbiological assay must be controlled carefully.
5. The use of standard culture medium in the test is one of the important steps for obtaining good results.

Directions

1. Suspend 42 grams of the medium in one liter of purified / distilled water containing 10ml of polysorbate 80.
2. Mix thoroughly and heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. After cooling, mix well and pour into sterile petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Yellow coloured slightly opalescent gel forms in Petri plates
Reaction of 3.05% solution	pH 7.1 ± 0.1 at 25°C
Gel Strength	Firm, comparable with 1.2% Agar gel

Growth Promotion Test

As per Indian Pharmacopoeia

Expected Cultural Response: Cultural characteristics observed after an incubation at different temperatures for 24 hours.

Sr. No.	Organisms	Results to be achieved				
		Inoculum (CFU)	Growth	Recovery	Antibiotics Assayed	Incubation Temperature
1.	*Bordetella bronchiseptica ATCC 4617*	50-100	luxuriant	>=50%	Polymyxin B, Colistimethate sodium, Colistin sulphate	32-35°C
2.	*Pseudomonas aeruginosa ATCC 27853*	50-100	luxuriant	>=70%	Carbenicillin	36-37.5°C
3.	*Escherichia coli ATCC 25922*	50-100	luxuriant	>=70%	Colistimethate sodium, Colistin sulphate	35-39°C

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

Test Procedure

Refer to appropriate standard references for details on testing protocol.

Results

Refer to appropriate references and 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.

Packaging
Product Name : Antibiotic Assay Medium H
Product Code : DM022I
Available Pack sizes : 100gm/500gm

References

1. Grove and Randall. 1955. Assay methods of antibiotics. Medical Encyclopedia, Inc. New York, N.Y.
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4. The United States Pharmacopoeia, 2009, The United States Pharmacopoeial Convention,Rockville, MD.
5. Abraham, Chain, Fletcher, Florey, Gardner, Heatley and Jennings. 1941. Lancett *ii*:177.
6. Foster and Woodruff. 1943. J. Bacteriol. *46*:187.
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8. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
9. Barry, 1991, Procedure and theoretical considerations for testing antimicrobial agents in agar media. Antibiotics in Laboratory medicine, New York pp 3 2.
10. Brown & Winsley, 1968. J Gen Microbiol. 1968 50(3) Suppl:ix.

Further Information

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