



PRODUCT SPECIFICATION SHEET

Antibiotic Assay Medium No.10 (Polymyxin Seed Agar) (DM022)

Intended Use

Antibiotic Assay Medium No.10 (Polymyxin Seed Agar) (DM022) is recommended to be used as a seed layer for the assay of products containing Polymyxin-B, also for assay of Carbenicillin, Colistin and Colistimethate sodium.

Product Summary and Explanation

Antibiotic assay media are prepared according to the specifications of the USP⁽¹⁾, European Pharmacopeia⁽²⁾ and AOAC International.⁽³⁾ The antibiotic media are identified numerically with names assigned by Grove and Randall in *Assay Methods of Antibiotics*.⁽⁴⁾ The activity (potency) of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms.⁽¹⁾ Reduction in antimicrobial activity may reveal changes not demonstrated by chemical methods.⁽¹⁾ 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⁾

This medium is used as seed agar for assay of Polymyxin B, Colistimethate sodium, Colistin and Carbenicillin. The medium composition is in accordance to USP and CFR.^(8, 9) To achieve satisfactory test results, the use of standardized culture media and careful control of all test conditions are fundamental requisites in the microbiological assay of antibiotics.

Principles of the Procedure

Antibiotic Assay Medium No. 10 contains a combination of pancreatic digest of casein and papaic digest of soybean provide essential nutrients for the growth of test organisms. Dextrose provides the carbon source, enhances the growth of test organism. Dibasic potassium phosphate in the medium enhances buffering action and sodium chloride maintains osmotic equilibrium. Polymixins are reported to have slow diffusion in agar giving smaller zone of inhibition.⁽¹⁰⁾ Hence the reduced agar concentration (1.2%) in this medium improves the diffusion of polymixin in the medium. Polysorbate 80 is reported to function synergistically with polymixins on spheroplasts of *Pseudomonas aeruginosa*. Polysorbate 80 enhances the penetration of Polymyxin to the cytoplasmic membrane and hence is an appropriate ingredient in the medium used for assay of Polymyxin.⁽¹¹⁾

Cylinder Plate Assay

This method is based on the diffusion of an antibiotic solution from a cylinder placed on the surface of an inoculated agar medium. After incubation, the diameter of a zone of inhibition is observed which depends, in part, on the concentration or activity of the antibiotic. The results depend on critical rates of diffusion of the antibiotic, critical growth rates of the standard organisms and critical minimal inhibitory coefficient levels of each organism. This method is used in the assay of commercial preparations of antibiotics, as well as in the quantitative determination of antibiotics in body fluids, animal feeds and other materials.

Pre-diffusion of antibiotics for 10-20 minutes in the agar by incubating at temperature below the optimal growth temperature for microorganism would facilitate better diffusion of antibiotics followed by incubation of plates for microbial growth.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	17.00
Papaic digest of soyabean meal	3.00
Sodium chloride	5.00
Dextrose	2.50
Dibasic potassium phosphate	2.50
Agar	12.00
Final pH: 7.2 ± 0.1	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

- For Laboratory Use only.





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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 distilled water containing 10 ml of Polysorbate 80.
2. Heat to boiling, to dissolve the medium completely, with frequent agitation to avoid charring.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Medium amber coloured clear to very slightly opalescent gel forms in Petri plates.
Reaction of 4.2% solution	pH 7.2 ± 0.1
Gel Strength	Firm, comparable with 1.2% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 32-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Antibiotics Assayed
1.	<i>Bordetella bronchiseptica</i> ATCC 4617	50-100	good-luxuriant	>=70%	Colistimethate Sodium, Colistin, Polymyxin B
2.	<i>Pseudomonas aeruginosa</i> ATCC 25619	50-100	good-luxuriant	>=70%	Carbenicillin

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

Test Procedure

Preparation of Stock cultures

1. Maintain stock cultures on agar slants and make transfers at 1- or 2-week intervals.
2. Using sterile purified water, saline or Antibiotic Medium 3, prepare the inoculum for assay by washing growth from a fresh 24-48 hour agar slant and further dilute the culture to obtain the desired organism concentration.
3. In some turbidimetric assays, an 18- 24hour culture of the test organism grown in Antibiotic Medium 3, diluted to obtain the optimal number of organisms, is used.

Cylinder Plate Assay

1. Use 20 × 100 mm glass or plastic Petri dishes with sufficient depth so that cylinders used in the assay will not be pushed into the medium by the cover.
2. Use stainless steel or porcelain assay cylinders having the following dimensions (± 0.1 mm): 8 mm outside diameter, 6 mm inside diameter and 10 mm long. Clean the cylinders carefully to remove all residues, using an occasional acid bath (i.e., with approximately 2N nitric acid or with chromic acid).
3. Four or six cylinders are generally used per plate, evenly spaced on a 2.8 cm radius.
4. For assuring accurate assays, use a level surface for working to obtain uniformly thick base and seed layers in the Petri dish.
5. Allow the base layer to solidify and then overlay the seed layer containing a proper concentration of the test organism. The amount of medium in the layers varies for different antibiotics, with most assays specifying a 21 mL base layer and a 4 mL seed layer.
6. In any case, dishes with flat bottoms are required to assure complete coverage of the bottom of the dish when small amounts of base medium are used. Tilt the plate to obtain even coverage of the base layer by the seed layer and allow it to solidify in a level position. Plates should be used the same day as prepared.

Results

Refer to appropriate references and specific test procedures.





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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

For a complete discussion of antibiotic assay methods, refer to appropriate procedures outlined in the references.^{1,2,3}

Packaging

Product Name : Antibiotic Assay Medium No.10 (Polymyxin Seed Agar)

Product Code : DMO22

Available Pack sizes : 100 gm/ 500gm

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp.1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. Council of Europe. 2002. European pharmacopeia, 4th ed. Council of Europe, Strasbourg, France.
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5. Abraham, Chain, Fletcher, Florey, Gardner, Heatley and Jennings. 1941. Lancett ii: 177.
6. Foster and Woodruff. 1943. J. Bacteriol. 46:187.
7. Schmidt and Moyer. 1944. J. Bacteriol. 47:199.
8. United States Pharmacopoeia / National Formulary 2011, US Pharmacopoeial Convention, Inc., Rockville, MD.
9. Tests and Methods of Assay of Antibiotics and Antibiotic containing Drugs, FDA, CFR, 1983 Title 21, Part 436, Subpart D, Washington, D.C.: U.S. Government Printing Office, paragraphs 436, 100-436, 106, p. 242-259, (April 1).
10. Barry, 1991, Procedure and theoretical considerations for testing antimicrobial agents in agar media. Antibiotics in Laboratory medicine, New York pp 3
11. Brown & Winsley, 1968.. J Gen Microbiol. 1968 50(3) Suppl:ix.

Further Information

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