Antibiotic Assay Medium No.6 (DM019)

Intended Use

Antibiotic Assay Medium No.6 (DM019) is recommended for induction of spore production in *Bacillus subtilis* strains used in antibiotic assays.

Product Summary and Explanation

Antibiotic assay media are prepared according to the specifications of the FDA,[1] USP[2], European Pharmacopeia[3] and AOAC International.[4] The antibiotic media are identified numerically with names assigned by Grove and Randall in *Assay Methods of Antibiotics*.[5] 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] Antibiotic assays are performed by the cylinder plate method and the turbidimetric "tube" assay.

Principles of the Procedure

Antibiotic Assay Medium No. 6 contains casein enzymic hydrolysate and papaic digest of soyabean meal provides the nutrients and growth factors for enhanced microbial growth. Dipotassium phosphate provides the buffering system. Manganese sulphate helps in the early initiation of *Bacillus* species. Dextrose stimulates the growth by providing carbon and energy. Sodium chloride maintains the osmotic equilibrium of the medium and retains the cell viability and cell integrity.

Turbidimetric Assay

The turbidimetric method is based on the change or inhibition of growth of a microbial culture in a liquid medium containing a uniform solution of an antibiotic. Turbidimetric determinations have the advantage of requiring a short incubation period, providing test results after 3 or 4 hours. However, the presence of solvents or other inhibitory materials may influence turbidimetric assays more markedly than cylinder plate assays. After incubation of the test organism in the working dilutions of the antibiotics, the amount of growth is determined by measuring the light transmittance using spectrophotometer. The concentration of antibiotic is determined by comparing amounts of growth obtained with that given by the reference standard solutions. Use of this method is appropriate only when test samples are clear.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	17.00
Papaic digest of soyabean meal	3.00
Sodium chloride	5.00
Dextrose	2.50
Dipotassium phosphate	2.50
Manganese sulphate	0.03
Final pH: 7.0 ± 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.
3. All conditions in the microbiological assay must be controlled carefully.
4. The use of standard culture medium in the test is one of the important steps for obtaining good results.

Directions

1. Suspend 30.03 grams of the medium in one liter of purified/ distilled water.
2. Heat if necessary to dissolve the medium completely.

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	Light amber coloured clear solution may contain slight precipitate
Reaction of 3.00% Solution	pH : 7.0 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed after an incubation at different temperatures for 6 days.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Incubated at	Spores
1.	*Bacillus cereus ATCC 10876*	50-100	good-luxuriant	30°C	positive
2.	*Bacillus stearothermophilus ATCC 7953*	50-100	good-luxuriant	55°C	positive
3.	*Bacillus subtilis ATCC 6633*	50-100	good-luxuriant	35°C	positive
4.	*Bacillus pumilus ATCC 14884*	50-100	good-luxuriant	35°C	positive

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 No. 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 No. 3, diluted to obtain the optimal number of organisms, is used.
4. For using as a test organism *Bacillus subtilis*, inoculate the organism on Antibiotic Medium No. 1 and incubate at 37°C for 1 week. Wash spores from the agar surface, and heat the spores at 56°C for 30 minutes. Using sterile purified water wash the spores three times, heat again at 65°C for 30 minutes, and then dilute to the optimal concentration. Inoculum prepared following this method should produce a sharp zone in the assay.
5. For preparing spore suspension of *B. subtilis,* Antibiotic Medium No. 1 modified by the addition of 300 mg manganese sulfate ($MnSO_4 \cdot H_2O$) per liter is used which aids in the sporulation of *B. subtilis*.

Turbidimetric Assay

1. Use glass or plastic test tubes (i.e., 16 × 125 mm or 18 × 150 mm) that are relatively uniform in length, diameter and thickness and substantially free from surface blemishes.
2. Tubes that will be placed in the spectrophotometer should be matched and free of scratches or blemishes.
3. Clean the tubes thoroughly to remove all antibiotic residues and traces of cleaning solution and, prior to subsequent use, sterilize tubes that have been previously used.
4. Prepare working dilutions of the antibiotic reference standards in specific concentrations.
5. To a 1 mL quantity of each solution in a suitable tube, add 9 mL of inoculated broth, as required.
6. Prepare similar solutions of the assay materials containing approximately the same amounts of antibiotic activity and place in tubes.
7. Incubate the tubes for 3-4 hours at the required temperature, generally in a water bath. At the end of the incubation period, stop growth by adding 0.5 mL of 1:3 formalin.
8. Determine the amount of growth by measuring light transmittance with a suitable spectrophotometer.
9. Determine the concentration of the antibiotic by comparing the growth obtained with that given by reference standard solutions.
10. Refer to appropriate procedures outlined in the references for a complete discussion of antibiotic assay methods.

Results

Refer to appropriate references and test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 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 No. 6
Product Code : DM019
Available Pack sizes : 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, Strasbourgh, France.
3. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
4. Grove and Randall. 1955. Assay methods of antibiotics. Medical Encyclopedia, Inc. New York, N.Y.
5. Rippere R. A.. Some principles of microbiological turbidimetric assays of antibiotics. J. Assoc. off. Anal. Chem. 1979. 62(4):951-6.

Further Information
For further information please contact your local MICROMASTER Representative.

MICROMASTER LABORATORIES PRIVATE LIMITED
Unit 38/39, Kalpataru Industrial Estate,
Off G.B. Road, Near 'R-Mall', Thane (W) – 400607. M.S. INDIA.
Ph: +91-9320126789/9833630009/9819991103
Email: sales@micromasterlab.com

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