



Antibiotic Assay Medium No.9 (Polymyxin Base Agar)

M147

Antibiotic Assay Medium No.9 (Polymyxin Base Agar) is used as base layer medium for assaying products containing Polymyxin B.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	17.000
Papaic digest of soyabean meal	3.000
Sodium chloride	5.000
Dipotassium phosphate	2.500
Dextrose	2.500
Agar	20.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 50 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

This medium is widely recommended for assay of Polymyxin B, Colistimethate sodium and Colistin using *Bordetella bronchiseptica* as test organisms. Carbenicillin assay is also performed using this medium with *Pseudomonas aeruginosa*. The medium is numerically identical with the name assigned by Groove and Randall (1).

Casein enzymic hydrolysate and papaic digest of soyabean meal serves as source for essential nutrients. Dextrose stimulates the growth by providing carbon and energy. Phosphates in the medium enhances buffering action and sodium chloride maintains osmotic equilibrium in the medium. Agar concentration provides control over the diffusion activity of polymyxin B antibiotics and provides solid substratum to support the seed agar layer.

To perform the antibiotic assay the Base Agar should be prepared on the same day as the test. For the cylinder method, a base layer of 21 ml is required. Once the base medium has solidified, seed layer inoculated with the standardized culture can be overlaid. Even distribution of the layer is important.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

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

Reaction

Reaction of 5% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

M147: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
<i>Bordetella bronchiseptica</i> ATCC 4617	50-100	good	50-70%	Colistimethate sodium,

<i>Pseudomonas aeruginosa</i> ATCC 25619	50-100	luxuriant	$\geq 70\%$	Colistin, Polymyxin B Carbenicillin
<i>Pseudomonas aeruginosa</i> ATCC 27853	50-100	luxuriant	$\geq 70\%$	Carbenicillin

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. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc. New York.

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