PM Indicator Agar (Penicillin in Milk Indicator Agar) M849

PM Indicator Agar (Penicillin in Milk Indicator Agar) is recommended for rapid detection of trace amounts of Penicillin in milk as per AOAC.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Casein enzymic hydrolysate	1.700
Papaic digest of soyabean meal	0.300
Beef extract	3.000
Dextrose	5.250
Sodium chloride	0.500
Dipotassium phosphate	0.250
Polysorbate 80	1.000
Bromo cresol purple	0.060
Agar	15.000
Final pH (at 25°C)	7.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 3.2 grams in 100 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and dispense as desired.

Principle And Interpretation

PM Indicator Agar is used for rapid detection of trace amounts of penicillin in milk where AOAC has recommended *Bacillus stearothermophilus* qualitative discs (1). PM Indicator Agar is designed according to the formula published by the 18th Annual Meeting of the National Mastitis Council (2). This method is a modification of the method approved by the International Dairy Federation for the qualitative detection of penicillin in milk (3). Originally a medium called Reductase Medium was suggested by Reid and Brewer for detecting penicillin in milk by using *Bacillus subtilis* (4). The present medium is better, faster and more reliable than that of Reid and Brewer (This medium is designed to support and demonstrate the growth and acid formation by *B. stearothermophilus* , which is sensitive to penicillin and b-lactam residues). To demonstrate the presence of traces of penicillin in milk; the qualitative disc method is found to be more suitable which is also recommended by AOAC.

Inoculate the medium with 1 ml of uniformly dispersed *B. stearothermophilus* suspension prepared as per AOAC (1). Prepare dilutions of standard penicillin G (positive control) to give concentrations in the range of 0.005 to 0.1 units/ml. Use a suspension of inhibitor free non-fat dry milk for negative control.

For Screening Assay : Place blank control disc, negative control disc, positive control disc on agar surface. Invert the plate and incubate till 17-20 mm zones of inhibition are obtained around positive control disc.

Interpretation of Screening Assay

Test Sample Positive Negative Interpretation Disc Control Disc Control Disc No zone Clear zone No zone Negative test indicating 17 - 20 mm that significant amounts of inhibiting substances are not present.

Zone Clear zone No zone Negative test indicating < 14mm 17-20 mm that significant amounts of inhibitory substances are not present.

Clear zone Clear zone No zone Positive test indicating > 14 mm 17-20 mm that an inhibitor is present in test sample. Perform CONFIRMING ASSAY to determine if inhibitor is a beta-lactam residue.

Any reaction Any reaction Any zone Result indicates error in the test system.Any reaction No zone Any reaction Determine source of error.

For Confirming Assay : Inactivate milk sample by heating at 82°C for 2 minutes. Cool promptly to room temperature. Invert the plate and incubate as in screening assay.

Interpretation of Confirming Assay

Penicillinase/ Interpretation Inactivated Positive Inactivated Test Control Disc Milk Sample Disc Sample DiscClear zone Clear zone No zone Positive test > 14 mm 17-20 mm indicating the presence of beta- lactam residues.

Clear zone Clear zone Clear zone, Positive test > 14 mm 17-20 mm same size as indicating the test sample presence of inhibitor(s) other than beta-lactam residues.

Clear zone Clear zone Clear zone Positive test > 14 mm 17-20 mm substan- indicating presence tially smaller of beta-lactam than 14 mm residues as well as other inhibitors.

Quality Control

Appearance
Beige to bluish grey homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Purple coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.2% w/v aqueous solution at 25°C. pH : 7.8±0.2

pH
7.60-8.00

Cultural Response
M849: Cultural response observed after an incubation in the following parameters...

Organism	Growth	Incubation temperature	Time
Bacillus stearothermophilus ATCC 7953	good	55 ± 2°C	3-4 hours
Bacillus stearothermophilus ATCC 7953	luxuriant	64 ± 2°C	3-4 hours

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. Williams, (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C
2. Publ. of the 18th Annual Meeting of Natl. Mastitis Council, Inc.
3. International Dairy Federation, 1970, International Dairy Federation, Brussels, Belgium.
4. Reid R. D. and Brewer J. H., 1946, J. Bacteriol., 52: 251.

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