M-PA-C AGAR

INTENDED USE

M-PA-C Agar is used for the selective recovery and enumeration of *Pseudomonas aeruginosa* from water samples by membrane filtration.

SUMMARY AND EXPLANATION

A variety of methods have been used for the enumeration of *P. aeruginosa* from water samples, some of which have been more widely accepted than others. The most-probable number (MPN) procedures result in satisfactory recovery levels of *P. aeruginosa*, but are not usable for the testing of large-volume water samples and lack precision. These two deficiencies are eliminated in membrane filter (MF) techniques.

Many of the membrane filter media used for the recovery of *P. aeruginosa* lacked specificity and were of limited value when large heterogeneous microbial flora were present in the water samples. Levin and Cabelli devised *M*-PA Agar as a selective membrane filter medium for *P. aeruginosa*. This formulation incorporated four antimicrobics, kanamycin, nalidixic acid, sulfapyridine and cycloheximide, which render the medium moderately selective. This original formulation was modified by raising the pH and altering the content or concentration of ingredients. The resulting medium was designated *M*-PA-B Agar. Brodsky and Ciebin further modified these media by eliminating sulfapyridine and cycloheximide and produced *M*-PA-C Agar. This formulation resulted in the ability to enumerate *P. aeruginosa* after only 24 hours of incubation at 41.5°C compared to 72 hours required with *M*-PA-B Agar and 96 hours for a presumptive MPN test. *M*-PA-C Agar is identified as Modified *M*-PA Agar in *Standard Methods for the Examination of Water and Wastewater*.

PRINCIPLE

Yeast extract, lysine and the carbohydrates provide carbonaceous and nitrogenous compounds, energy sources and vitamins required for bacterial metabolism. Sodium chloride maintains osmotic equilibrium. The salts provide essential ions. Phenol red is a pH indicator, which becomes yellow in response to acids produced as a result of the fermentation of the carbohydrates. Kanamycin inhibits protein synthesis in gram-positive organisms. Nalidixic acid blocks replication of susceptible gram-negative bacteria.

REAGENTS (FORMULA)

Yeast Extract	2.0	g
L-Lysine	5.0	g
Sodium Chloride	5.0	g
Xylose	1.25	g
Sucrose	1.25	g
Lactose	1.25	g
Phenol Red	0.08	g
Ferric Ammonium Citrate	0.8	g
Sodium Thiosulfate	5.0	g
Magnesium Sulfate	1.5	g
Kanamycin	0.008	g
Nalidixic Acid	0.037	g
Agar	12.0	g
Deionized Water	1000.0	ml

PROCEDURE

Following filtration of the water sample through a sterile 47 mm, 0.45 µm gridded filter, place the membrane filter on the surface of a plate of *M*-PA-C Agar taking care to avoid the entrapment of bubbles between the agar and filter surface. Incubate for 40-48 hours (until 72 hours is acceptable) at 41.5 ± 0.5°C in an aerobic atmosphere. Consult the standard method for additional information regarding the *M*-PA-C membrane filter technique.

EXPECTED RESULTS

Colonies on membrane filters are counted using a stereoscopic microscope at 10-15× magnification. Optimal colony density is 20-80 colonies. All colonies on the filter are counted when the density is 2 or fewer per square. The average of 10 squares is determined when the count is 3-10 colonies per square and the average of 5 squares is determined when the count is 10-20 colonies per square. Multiply the average count per square by 100 and divide by the sample volume to give colonies per milliliter.

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
Pseudomonas aeruginosa ATCC 27853	41.5 ± 0.5°C for 40-48 hours	Growth; no color change
Escherichia coli ATCC 25922	41.5 ± 0.5°C for 40-48 hours	Partial to complete inhibition
Proteus mirabilis ATCC 12453	41.5 ± 0.5°C for 40-48 hours	Partial to complete inhibition; no swarming

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CBSA life

155-196 Innovation Drive, Winnipeg, MB, R3T 2N2, Canada

Phone: +1 (204) 269-2255 Email: info@cbsalife.com Website: https://cbsalife.com