



Milk Agar (Brown and Scott Modified)(Twin Pack)

M782

Milk Agar (Brown and Scott Modified) (Twin Pack) is used for the confirmation of *Pseudomonas aeruginosa* in swimming pool waters.

Composition**

Ingredients	Gms / Litre
Part A	-
Instant non-fat milk	100.000
Part B	-
Peptic digest of animal tissue	5.000
Sodium chloride	5.000
Beef extract	1.500
Yeast extract	1.500
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Part A: Suspend 100 grams in 500 ml distilled water. Sterilize by autoclaving at 15 lbs pressure (121°C) for 5 minutes. Cool to 55°C.

Part B: Suspend 28 grams in 500 ml distilled water and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool rapidly to 55°C. Mix Part A and Part B together and pour into sterile Petri plates.

Principle And Interpretation

Milk Agar was modified by Brown and Scott (1) for the confirmation of *Pseudomonas aeruginosa* in swimming pool waters. Swimming pool water is generally chlorinated potable water but it can also be from thermal springs or salt water. Microorganisms of concern are typically those from the body of the bather's including the arifices. *Pseudomonas aeruginosa* is one of the major supporting indicator organisms in the swimming pool. This organism is mainly responsible for ear and eye infection and is very likely to get disseminated in the swimming pool water due to constant contact of ears and eyes with the water.

Milk, peptic digest of animal tissue, yeast extract, beef extract provide all the necessary nutrients mainly nitrogenous for the multiplication of *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* forms yellowish green colonies on this medium.

P. aeruginosa hydrolyzes casein and produces a yellowish to green diffusible pigment on Milk Agar. For isolation, filter 200 ml or less water of the swimming pool through sterile membrane filters. Place each membrane filter on M-PA Agar (M1121). Incubate the plates at 41.5±0.5°C for 72 hours. Typical *P. aeruginosa* colonies are 0.8-2.2 mm in diameter, flat in appearance with brownish to greenish centers. For confirmation, using Milk Agar, make a single streak from an isolated colony on a Milk Agar plate and incubate at 35-37°C for 24 hours. After incubation *P. aeruginosa* forms pigmented colonies.

Quality Control

Appearance

Part A : Cream to off white homogeneous free flowing powder Part B - Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light amber coloured opalescent gel forms in Petri plates

Reaction

Reaction of 2.8% w/v aqueous solution of Part B at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

M782: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Pigment Production
<i>Escherichia coli</i> ATCC 25922	50-100	good-luxuriant	no pigment
<i>Pseudomonas aeruginosa</i> ATCC 27853	50-100	good-luxuriant	yellowish green

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C and use as fresh as possible. Use before expiry date on the label.

Reference

1. Brown M.R.W. and Scott F. J.H., 1970, J. Clin. Pathol., 23:172.

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