Technical Information

Milk Agar with Cetrimide (Twin Pack)

Product Code: DM 2273

Application: - Milk Agar with Cetrimide is used for the detection and enumeration of *Pseudomonas aeruginosa* in swimming pool waters.

Composition**

Ingredients	Gms / Litre
Part A	133.330
Skim milk powder	-
Part B	3.330
Peptic digest of animal tissue	1.670
Sodium chloride	1.000
Yeast extract	0.400
Cetrimide	20.000
Agar	7.3±0.2
Final pH (at 25°C)	

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Milk Agar was modified by Brown and Scott [1] for isolation & 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 from the body of the bathers, including the orifices. As *P.aeruginosa* can survive for longer time in water compared to other microorganisms, it is one of the major indicator organisms for detecting contamination 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 of bathers with the water.

Milk Agar with Cetrimide is formulated in accordance with ISO Committee under the specifications ISO 8360-1: 1988 for the detection and enumeration of *P.aeruginosa* from water [2]. Strains of *P.aeruginosa* are gram negative rod and identified by their pigment production i.e. pyocyanin is the only species of *Pseudomonas to* excrete pyocyanin.

P.aeruginosa hydrolyzes casein and produces a yellowish to green diffusible pigment on Milk Agar. For isolation, filter 200ml or less water of the swimming pool through sterile membrane filters. Place each membrane filter on M-PA Agar (DM2121). 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 w/ Cetrimide, make a single streak from an isolated colony on a Milk Agar w/ Cetrimide plate and incubate at 35-37°C for 24 hours. After incubation *P.aeruginosa* forms pigmented colonies.

Milk, skim milk powder, peptic digest of animal tissue and yeast extract provide all the necessary nutrients mainly nitrogenous for the multiplication of *P.aeruginosa.*

P.aeruginosa forms yellowish green colonies on this medium. Cetrimide acts as a quaternary ammonium, cationic detergent that causes release of nitrogen and phosphorus from bacterial cells other than *P.aeruginosa.*

Methodology

Suspend 26.4 grams of powder media of Part B in 250 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 20 minutes. Suspend 133.33 grams of Part A in 750 ml of distilled water and sterilize by autoclaving at 15 lbs pressure (121°C) for 5 minutes. After autoclaving mix Part A and B and pour into sterile Petri plates.

Quality Control

Physical Appearance
Part A : White to cream homogeneous free flowing powder Part B : Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 2.0%

Colour and Clarity of prepared medium
Light amber coloured opalescent gel forms in petri plates.

Reaction
Reaction of 2.68% w/v aqueous solution of Part B at 25°C. pH : 7.3±0.2

pH range 7.10-7.50

Cultural Response/Characteristics
DM2273: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Pigment
Escherichia coli ATCC 25922	$>10^3$	Inhibited	--
Pseudomonas aeruginosa ATCC 27853	50-100	Good-Luxuriant	Blue green
Stenotrophomonas maltophilia ATCC 13637	$>10^3$	Inhibited	--

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Brown M. R. W. and Scott F. J. H., 1970, J. Clin. Pathol., 23:172.
2. International Organization for Standardization (ISO), Draft ISO/DIS 8360-1:1988.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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