



Motility Nitrate Medium, Buffered

M630

Motility Nitrate Medium, Buffered is recommended for isolation and detection of *Clostridium perfringens* on the basis of motility and nitrate test.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Beef extract	3.000
Galactose	5.000
Potassium nitrate	1.000
Disodium phosphate	2.500
Agar	3.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 19.5 grams in 1000 ml distilled water containing 5 ml glycerol. Heat to boiling to dissolve the medium completely. Dispense in test tubes to make them half full. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool quickly in cool running water and allow the tubed medium to solidify in an upright position.

Principle And Interpretation

Clostridium perfringens food poisoning is one of the most common type of human foodborne illness. The foods usually involved are cooked meat or poultry products containing large number of viable cells. *Clostridium perfringens* is a gram-positive, rod shaped anaerobic, spore-forming bacteria that produces enterotoxin. This toxin if ingested, can cause food poisoning. Motility Nitrate Medium, Buffered formulated in accordance with FDA (1) and APHA (2), is recommended for the detection of *C.perfringens* on the basis of motility and nitrate test.

Peptic digest of animal tissue and beef extract supply amino acids and other complex nitrogenous substances. Agar is added to obtain a semisolid gel that helps to demonstrate motility of the organism along the stab line of inoculation. Growth of motile organisms extends out from the line of inoculation. The medium contains 0.5% each of glycerol and galactose to improve the consistency of the nitrate reduction reaction with different strains of the organisms (3). Potassium nitrate serves as a base for nitrate reduction. A red or orange colour formation on addition of nitrate reagents indicates reduction of nitrate to nitrite.

Motility is indicated by turbidity extending out from the line of stab inoculation. Non-motile organisms grow only in the inoculated area. After 3-8 hours of incubation, a small puffball of motility may be seen around the line of inoculation. If this is not observed, tubes should be re-incubated for 24-48 hours and compared for turbidity to an un-inoculated tube. Negative motility reactions should be confirmed by a hanging drop preparation.

In the nitrate reduction test, a pink to red color develops after addition of the reagents if nitrite is present. Colour development indicates that nitrate reduction has occurred in the tube. Some organisms further reduce nitrite to ammonia that can be detected by the addition of a small amount of zinc dust to the tubes exhibiting no colour. A pink colour in this part of the test indicates no nitrate reduction. A colourless reaction indicates that nitrates have been completely reduced.

Inoculate 2 grams of food sample in 15 to 20 ml of Chopped Liver Broth (M606) or Tryptone Glucose Yeast Extract Broth (M952). After an incubation at 35-37°C for 20-24 hours, isolate on Perfringens Agar Base (TSC/SFP Agar Base) (M837). Presumptive *C.perfringens* colonies are confirmed biochemically by inoculating into Motility Nitrate Medium, Buffered to detect motility and nitrate reduction.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.3% Agar gel.

Colour and Clarity of prepared medium

Light amber coloured clear to slightly opalescent gel forms in tubes as butts

Reaction

Reaction of 1.95% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

M630: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours .

Organism	Inoculum (CFU)	Growth	Motility	Nitrate reduction
Cultural Response <i>Clostridium absonum</i> ATCC 27555	50-100	luxuriant	weakly motile	weak or negative reaction
<i>Clostridium perfringens</i> ATCC 12924	50-100	luxuriant	negative, growth along the stabline, surrounding medium remains clear	positive, red-violet colour developed within 1-2 minutes

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. Bacteriological Analytical Manual, Food and Drug Administration, 1995, 8th Ed., AOAC International, Gaithersburg, Md., USA.
2. Downes F. P. and Ito K., (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.

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