



PRODUCT SPECIFICATION SHEET

Motility Nitrate Medium, Buffered (DM399)

Intended Use

Motility Nitrate Medium, Buffered is recommended for isolation and detection of *Clostridium perfringens* on the basis of motility and nitrate test. The composition and performance criteria of this medium are as per the specifications laid down in ISO 7937:1985.

Product Summary and Explanation

The foods usually involved are cooked meat or poultry products containing large number of viable cells. *Clostridium perfringens* food poisoning is one of the most common type of human foodborne illness. *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. Motility Nitrate Medium, Buffered with a slightly lower pH is recommended by the ISO Committee for isolation and detection of *C. perfringens* on the basis of motility and nitrate test ⁽³⁾. 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.

Principles of the Procedure

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.

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.

Formula / Liter

Ingredients	Gms / Litre
Peptic digest animal tissue	5.00
Beef extract	3.00
Galactose	5.00
Potassium nitrate	1.00
Disodium phosphate	2.50
Agar	3.00
Final pH: 7.1 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

- For Laboratory Use only.
- IRRITANT, mainly irritating to eyes, respiratory system, and skin.





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Directions

1. Suspend 19.5 grams of the medium in one liter of distilled water/purified water containing 5ml glycerol.
2. Heat to boiling to dissolve the medium completely.
3. Dispense into tubes to make them half full, and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
4. Cool quickly in cool running water and allow the tubed medium to solidify in a upright position.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light amber coloured clear to slightly opalescent gel forms in tubes as butts
Reaction of 2.0% Solution	pH 7.4 ± 0.2 at 25°C
Gel Strength	Semisolid, comparable with 0.3% Agar gel

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

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Motility	Nitrate reduction
1.	<i>Clostridium absonum</i> ATCC 27555	50-100	luxuriant	Weekly Motile	weak or negative reaction
2.	<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

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate 2 grams of food sample in 15 to 20 ml of Chopped Liver Broth (DM1487) or Tryptone Glucose Yeast Extract Broth (DM386). After an incubation at 35-37°C for 20-24 hours, isolate on Perfringens Agar Base (TSC/SFP Agar Base) (DM566). Presumptive *C.perfringens* colonies are confirmed biochemically by inoculating into Motility Nitrate Medium, Buffered to detect motility and nitrate reduction.

Results

1. 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.
2. 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.

Storage

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

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Many organisms fail to grow deep in semisolid media; inoculating pour plates may be advantageous.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Motility Nitrate Medium Buffered

Product Code : DM399

Available Pack sizes : 100gm / 500gm





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References

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.
3. International Organization for Standardization (ISO), 1985, Draft ISO/DIS 7937

Further Information

For further information please contact your local MICROMASTER Representative.



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