

BBL™ Prepared Tubed Medium for Differentiation of Gram-Negative Bacilli

Motility Nitrate (MN) Medium
(Pickett's, for Nonfermenters)

Pkg. of 10 size E tubes

Cat. No. 296309

INTENDED USE

Motility Nitrate (MN) Medium (Pickett's, for nonfermenters) is used for the detection of motility, nitrate reduction and denitrification of nonfermenting gram-negative bacilli.

SUMMARY AND EXPLANATION

Motility Nitrate (MN) Medium is based on the formulation of M.J. Pickett.^{1,2} Microorganisms may be differentiated according to their metabolism of certain substrates. The ability to reduce nitrate to nitrite is characteristic of the family *Enterobacteriaceae*.³ Nonfermenters and other miscellaneous gram-negative bacilli vary in their ability to reduce nitrates. Some members of this group are capable of denitrification, which is a reduction of nitrate to nitrogen gas. The production of gas from nitrate is an important differential test for glucose-nonfermenting gram-negative bacilli. The end product of reduction depends upon the bacterial species.⁴

The medium is also used for indirect evidence of motility by nonfermenting gram-negative bacilli.

PRINCIPLES OF THE PROCEDURE

Enzymatic digests of casein and heart infusion supply amino acids and other complex nitrogenous substances. Yeast extract supplies the B-complex vitamins. Agar is added to demonstrate motility of the organism along a stab line of inoculation. Growth of motile organisms extends out from the line of inoculation.

Potassium nitrate is added to detect those nonfermenting gram-negative rods that can reduce nitrate to nitrite. Some organisms can further reduce the nitrite to nitrogen gas. Detection of denitrification activity can be useful for the identification of isolates, since only a small number of species of nonfermenting gram-negative rods can denitrify.⁵

REAGENTS

Formula:

Approximate Formula* Per Liter Purified Water

Heart Muscle, Infusion (from solids)	0.4 g
Pancreatic Digest of Casein	9.1
Yeast Extract	4.5
Sodium Chloride	1.0
Agar	3.0
Potassium Nitrate	1.0

* Adjusted and/or supplemented as required to meet performance criteria.

Precautions: *in vitro* Diagnostic

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes and other contaminated materials must be sterilized by autoclaving.

Storage Instructions: On receipt, store tubes in the dark at 2 to 25°C. Avoid freezing and overheating. Allow the medium to warm to room temperature before inoculation. Do not incubate prior to use. Do not open until ready to use. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times.

Product Deterioration: Do not use tubes if they show evidence of contamination, discoloration, evaporation, precipitation or other signs of deterioration.

SPECIMEN COLLECTION AND TRANSPORT

These media are not suitable for use directly with specimens or other materials containing mixed microbial flora. Consult references for information.^{6,7}

PROCEDURE

Material Provided: Motility Nitrate (MN) Medium

Materials Not Provided: Ancillary culture media, reagents, quality control cultures and laboratory equipment as required for this procedure.

Instructions: Loosen caps, place tubes in boiling water and cool to room temperature before use.

Organisms to be cultivated must first be isolated in pure culture on an appropriate solid medium.

Using a sterile straight, smooth inoculating needle, remove growth from the subculture medium and stab the center of the motility medium to 5 to 10 mm in depth.

Incubate tubes at 18 to 20°C.⁷

Tubes may be examined for motility after 3 to 8 h. If tubes are negative, reincubate and examine again after 24 to 48 h. The opacity of the medium should be compared to an uninoculated tube.

To test for nitrate reduction (on cultures incubated 42 to 48 h):

- Add 0.5 mL of sulfanilic acid solution.
- Add 0.5 mL of N,N-dimethyl-1-naphthylamine solution.
- Observe for the production of a pink to red color (positive nitrate reduction test). Since some organisms further reduce nitrite to ammonia, add a small amount of zinc dust to tubes exhibiting no color. A pink color in this part of the test indicates no nitrate reduction, whereas, a colorless reaction indicates that nitrates have been completely reduced.

User Quality Control:

- Examine the tubes for signs of deterioration as described under "Product Deterioration."
- Check performance by inoculating a representative sample of tubes with pure cultures of stable control organisms that produce known, desired reactions. The following cultures are recommended:

TEST STRAIN	EXPECTED RESULTS	
	Motility	Nitrate Reduction
<i>Acinetobacter baumannii</i> ATCC® 19606	—	—
<i>Achromobacter xylosoxidans</i> subsp. <i>denitrificans</i> ATCC 15173	+	+

RESULTS

Motility is indicated by turbidity extending out from the line of stab inoculation. Nonmotile organisms grow only in the inoculated area. After 3 to 8 h of incubation, a small puffball of motility may be seen around the line of inoculation.⁵ If this is not observed, tubes should be reincubated for 24 to 48 h and compared for turbidity to an uninoculated tubes. 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, and indicates that nitrate reduction has occurred. Since some organisms further reduce nitrite to ammonia, add a small amount of zinc dust to tubes exhibiting no color. A pink color in this part of the test indicates no nitrate reduction. A colorless reaction indicates that nitrates have been completely reduced.

Consult appropriate references for an explanation of the reactions involved and expected results with specific microorganisms.^{2,5,8}

LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Negative motility reactions should be confirmed by a hanging drop preparation. Morphological, biochemical and/or serological tests should be performed for final identification. Consult appropriate references for detailed information and recommended procedures.^{2,5-8}

REFERENCES

1. Blachman, U., and M.J. Pickett. 1978. Unusual aerobic bacilli in clinical bacteriology. Scientific Developments Press, Los Angeles.
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5. Gilardi, G.L. (ed.). 1985. Nonfermentative gram-negative rods. Marcel Dekker, Inc., New York.
6. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis.
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8. Gilardi, G.L. 1988. Identification of glucose-nonfermenting gram-negative rods. Department of Laboratories, North General Hospital, New York.

TECHNICAL INFORMATION: In the United States, telephone Technical Services, toll free (800) 638-8663.



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