

- c. 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 (Cat. No. 261207) to tubes exhibiting no color.

Expected Results

Motility is indicated by turbidity extending out from the line of stab inoculation. Nonmotile organisms grow only in the inoculated area. After 3-8 hours of incubation, a small puff-ball of motility may be seen around the line of inoculation.⁵ If this is not observed, tubes should be reincubated for 24-48 hours and compared for turbidity to an uninoculated 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, and indicates that nitrate reduction has occurred. Since some organisms further reduce nitrite to ammonia, add a small amount of zinc dust (Cat. No. 261207) 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.⁶⁻⁸

References

- Blachman and Pickett. 1978. Unusual aerobic bacilli in clinical bacteriology. Scientific Developments Press, Los Angeles, Calif.
- Pickett. 1980. Nonfermentative gram-negative bacilli. Scientific Developments Press, Los Angeles, Calif.
- Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., New York, N.Y.
- MacFaddin. 2000. Biochemical tests for the identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
- Gilardi (ed.). 1985. Nonfermentative gram-negative rods. Marcel Dekker, Inc., New York, N.Y.
- Gilardi. 1988. Identification of glucose non-fermenting gram-negative rods. Dept. of Laboratories, North General Hospital, New York, N.Y.
- Forbes, Sahm and Weissfeld. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis, Mo.
- Murray, Baron, Pfaller, Tenover and Tenover (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Motility Nitrate (MN) Medium

Cat. No. 296309 Prepared Tubes (K Tubes) – Pkg. of 10*

Difco™/BBL™ Nitrate A Reagent

Cat. No. 261197 Droppers, 0.5 mL – Ctn. of 50

Difco™/BBL™ Nitrate B Reagent

Cat. No. 261198 Droppers, 0.5 mL – Ctn. of 50

Difco™/BBL™ Nitrate C Reagent

Cat. No. 261207 Droppers, 1 g – Ctn. of 50

*Store at 2-8°C.

Motility Test Medium

Intended Use

Motility Test Medium is used for the detection of motility of gram-negative enteric bacilli.

Summary and Explanation

In 1936, Tittsler and Sandholzer reported on the use of semisolid agar for the detection of bacterial motility.¹ Their original formulation has been modified in the medium supplied as BBL™ brand Motility Test Medium.

Principles of the Procedure

Bacterial motility can be observed directly from examination of the tubes following incubation. Growth spreads out from the line of inoculation if the organism is motile. Highly motile organisms provide growth throughout the tube. Growth of nonmotile organisms only occurs along the stab line.

Formula

BBL™ Motility Test Medium

Approximate Formula* Per Liter	
Beef Extract	3.0 g
Pancreatic Digest of Casein	10.0 g
Sodium Chloride	5.0 g
Agar	4.0 g

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

Directions for Preparation from Dehydrated Product

- Suspend 22 g of the powder in 1 L of purified water. Mix thoroughly.
- Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- Dispense and autoclave at 121°C for 15 minutes.
- If desired, 5 mL of sterile 1% TTC solution may be added aseptically after autoclaving.
- Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate tubes with a pure culture by stabbing the center of the column of medium to greater than half the depth. Incubate tubes for 24-48 hours at 35 ± 2°C in an aerobic atmosphere.

Expected Results

After incubation, observe the tubes for growth in relation to the stab line. Nonmotile organisms grow only along the line of inoculation, while motile organisms spread out from the line of inoculation and may even grow throughout the medium.

Negative tubes can be reincubated at 25 ± 2°C for an additional 5 days, if desired.

Consult appropriate texts for results with specific organisms.^{2,3}

User Quality Control

Identity Specifications

BBL™ Motility Test Medium

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.2% solution, soluble in purified water upon boiling. Solution is pale to light, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Pale to light, yellow to tan, clear to slightly hazy.
Reaction of 2.2% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ Motility Test Medium

Prepare the medium per label directions. Stab inoculate with fresh cultures and incubate at 35 ± 2°C for 2 days.

ORGANISM	ATCC™	RECOVERY	MOTILITY
<i>Enterobacter aerogenes</i>	13048	Good	+
<i>Escherichia coli</i>	25922	Good	+
<i>Klebsiella pneumoniae</i>	33495	Good	-
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	Good	+
<i>Shigella flexneri</i>	9199	Good	-
<i>Proteus vulgaris</i>	8427	Good	+



Limitation of the Procedure

Many organisms fail to grow deep in semisolid media; inoculating pour plates may be advantageous.⁴

References

- Tittsler and Sandholzer. 1936. *J. Bacteriol.* 31:575.
- Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. *Bergey's Manual™ of determinative bacteriology*, 9th ed. Williams & Wilkins, Baltimore, Md.
- Farmer. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), *Manual of clinical microbiology*, 7th ed. American Society for Microbiology, Washington, D.C.
- MacFaddin. 1985. *Media for isolation-cultivation-identification-maintenance of medical bacteria*, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Motility Test Medium

BAM	COMPF	
Cat. No.	211436	Dehydrated – 500 g
	221509	Prepared Tubes – Pkg. of 10
	221510	Prepared Tubes – Ctn. of 100

Difco™ TTC Solution 1%

Cat. No.	231121	Tube – 30 mL
	264310	Bottle – 25 g

Mucate Agar • Mucate Broth

Intended Use

These media are used in the differentiation of certain *Enterobacteriaceae* based on utilization of mucate.

Summary and Explanation

Kauffman and Peterson devised mucate medium (broth) for differentiation of some members of the *Enterobacteriaceae* based on their ability to utilize mucate as a source of carbon.¹ Mucate Agar is a solid form of mucate medium. Utilization of the mucate produces an acid reaction, which causes the medium to become yellow. Ellis et al. recommended using mucate medium and two other organic acid media (D-tartrate and sodium citrate) in conjunction with other tests for differentiation among *Salmonella* and *Arizona* strains.² Mucate utilization is also recommended for differentiation of other genera of the *Enterobacteriaceae*, such as *Escherichia coli* and *Shigella* species.³⁻⁶

Principles of the Procedure

Gelatin peptone provides amino acids and other nitrogenous substances to support bacterial growth.

Mucate, which is produced from mucic acid when the pH is adjusted with sodium hydroxide during manufacture of the medium, is the sole source of carbon. Utilization of the mucate results in an acid reaction, which lowers the pH of the medium and causes the bromthymol blue indicator to change the color of the medium from blue-green to yellow. The medium remains blue-green if the organism being tested does not utilize the mucate.

Mucate utilization is intended to be used in conjunction with other tests for differentiation and identification of certain members of the *Enterobacteriaceae*.