MANUAL OF NONFERMENTING GRAM - NEGATIVE BACTERIA

Thomas R. Oberhofer, DrPH

MANUAL OF NONFERMENTING GRAM-NEGATIVE BACTERIA

Thomas R. Oberhofer, DrPH
Chief, Microbiology Section
Department of Pathology and Area Laboratory Services
Department of the Army
Madigan Army Medical Center
Tacoma, Washington

A WILEY MEDICAL PUBLICATION
JOHN WILEY & SONS
New York · Chichester · Brisbane · Toronto · Singapore

Thomas R. Oberhofer, DrPH, is a member of the U.S. Army. This manual was written by Dr. Oberhofer in his private capacity and no official support or endorsement by the U.S. Army or by the Army Medical Department is intended or implied.

Library of Congress Cataloging in Publication Data:

Oberhofer, Thomas R.

Manual of nonfermenting gram-negative bacteria.

(A Wiley medical publication)

1. Gram negative bacteria—Identification—Handbooks, manuals, etc. 2. Diagnosis, Laboratory—Handbooks, manuals, etc. I. Title. II. Series.

QR67.0238 1985 616.07'581 84-21965

ISBN 0-471-80544-0

PREFACE

The *Manual of Nonfermenting Gram-Negative Bacteria* has been prepared to serve those laboratory workers, or those taking courses in diagnostic microbiology, for whom only a minimum of text or workbook reference material is available. The manual is for the use of laboratory workers who are advanced in the study of bacteriology, or at least are beyond the student-trainee stage, and who wish to consider seriously identification of nonfermenting bacteria. The student or novice will find the manual valuable for the detailed explanation of media selection and test performance.

In the past 15 years, great strides have been made in the recognition, identification, and characterization of the nonfermenting bacteria. These advances have resulted from clarification of the nomenclature and taxonomy of the nonfermenters, and from which a systematic approach to their identification is now possible. However, media and test conditions used to identify and differentiate the nonfermenters are diverse and tables of identification are fragmented in their constituency. Moreover, tables of identification in much of the published literature are compilations of results obtained by different methodologies. The choice of each test and mode of identification, then, presents a continuous challenge to the laboratory worker.

This manual represents my experiences with nonfermenting bacteria and includes methods that I have used successfully for identifying and differentiating clinical isolates. While it is not my intention to dictate which tests must or ought to be used, it is clear that certain media and methods have withstood intense scrutiny whereas others are still subject to sustained evaluation. I have included data that have resulted from my use of these test methods. It is my hope that the user of this manual will find these methods useful in identifying the nonfermenting bacteria.

Thomas R. Oberhofer

LIST OF TESTS AND PROCEDURES

PART 1. METHODS

PART 1
METHODS

ACETAMIDE UTILIZATION

DESCRIPTION

Many nonfermentative bacteria produce an acylamidase that, when acting on acetamide, deamidates the acetamide to release ammonia into the supporting medium. The test for utilization of acetamide is comparable to the test for utilization of citrate in that acetamide is incorporated into Simmons agar base at a concentration of 1%. Utilization is evidenced by a change of the bromothymol blue indicator from green (neutral) to blue (alkaline). The acetamide test is particularly powerful because it selects out certain organisms within the nomenclatural group of nonfermenters.

TEST PROCEDURE

1. Inoculate an acetamide slant with one drop of a broth or saline suspension of the test organism, and allow the drop to run down the slant. Alternatively, use a straight wire to select a portion of an isolated colony and streak the slant.
2. Incubate the slant overnight at 35°C with the cap loose.
3. Reincubate those tubes showing no reaction for an additional 24 hours. Tests should be held for 7 days if possible.

INTERPRETATION

1. A positive test, indicating utilization of acetamide, is characterized by a change in the indcator color from green to stark blue.
2. A negative test is seen as no growth, or slight growth, but there is no change in the indicator.
3. A slight discoloration of the slant is ignored.

NOTES

1. The test for acetamide acylamidase (a deamidase) is more correctly termed a test for alkalinization rather than for utilization, since some organisms may grow slightly on the slant but casue no color change. *Pseudomonas maltophilia* is particularly noteworthy in this respect.
2. The acetamide test is suitable to replace the test for growth at 42°C for the routine differentiation of the fluorescent pseudomonads. *P. aeruginosa* attacks acetamide and grows at 42°C, whereas the other fluorescent pseudomonads do not.

REFERENCES

Bühlmann, X, W. A. Vischer, and H. Bruhin. 1961. Identification of apyocyanogenic strains of *Pseudomonas aeruginosa*. *J. Bacteriol.* 82: 787.

Hedberg, M. 1969. Acetamide agar medium selective for *Pseudomonas aeruginosa*. *Appl. Microbiol.* 17: 481.

Oberhofer, T. R. 1979. Growth of nonfermentative bacteria at 42°C. *J. Clin. Microbiol.* 10: 800.

Oberhofer, T. R. and J. W. Rowen. 1974. Acetamide agar for differentiation of nonfermentative bacteria. *Appl. Microbiol.* 28: 720.

Oberhofer, T. R., J. W. Rowen, and G. F. Cunningham. 1977. Characterization and identification of Gram-negative, nonfermentative bacteria. *J. Clin. Microbiol.* 5: 208.

AMIDE AGAR MEDIUM

1. *Formula*

   (a) *Recommended*

$MgSO_4$	0.2 g
$NH_4H_2PO_4$	1.0 g
K_2HPO_4	1.0 g
NaCl	5.0 g
Agar	15.0 g
Bromothymol blue	0.08 g

   (b) *Alternate*

$MgCl \cdot 6H_2O$	0.2 g
KH_2PO_4	1.0 g
K_2HPO_4	1.0 g
NaCl	5.0 g
Agar	15.0 g
Bromothymol blue	0.08 g

   (c) *Bromothymol blue indicator.* Dissolve 0.1 g of the powder in 8 ml of 50% *N* NaOH. Add 6.4 ml of the indicator solution to each liter of base medium.

2. *Preparation*

   (a) Add the ingredients to 1000 ml of deionized water. Heat to dissolve.

Table 1. Alkalinization of Acetamide, Allantoin, and Tartrate

Organism	No. Tested	Acetamide +(+)[a]	Acetamide %+	Allantoin +(+)	Allantoin %+	Tartrate +(+)	Tartrate %+
P. aeruginosa							
Apyocyanogenic	218	212(5)	99	108(62)	78	0(1)	1
Pyomelanine	35	33	100	9(9)	51	0	0
Delayed pyocyanin	50	50	100	27(13)	80	0	0
Mucoid	43	43	100	8(25)	77	0	0
Nonglucolytic	7	1(3)[a]	57	0	0	0	0
UFP-1 and -2[b]	16	0	0	0	0	0	0
P. putida	178	2(1)	2	3(2)	3	40(4)	25
P. fluorescens	105	0(1)	1	4(8)	11	6	6
P. pseudomallei	6	0(2)	33	0	0	0	0
P. stutzeri group	54	1(7)	13	0	0	4	7
P. pseudoalcaligenes	29	0(1)	3	0	0	0	0
A. anitratus	357	7(4)	3	4(21)	7	117(19)	38
A. haemolyticus	28	0	0	0(1)	4	4	16
P. thomasii	3	0	0	2(0)	100	0	0
P. picketii	13	0	0	12(1)	100	13	100
CDC Va-1	17	0	0	14(1)	88	16(1)	100
Pseudomonas							
sp.1	10	0	0	10	100	0	0
sp.2	7	6	0	0	0	0	0
A. xylosoxidan							
bio 1	46	31(13)	96	9(2)	24	0(1)	2
bio 2	6	0(2)	33	1	17	0	0
Achromobacter							
sp.1	10	0	0	9(1)	100	0	0
sp.2	4	0	0	2(2)	100	0	0
CDC Vd-1	13	0	0	12(1)	100	0(1)	8
CDC Vd-2	3	0	0	3	100	0	0
P. maltophilia	223	1(9)	4	0	0	1(8)	4
P. cepacia	36	20(1)	58	11(1)	33	35(1)	100
F. meningosepticum	10	0(1)	10	0(1)	10	0	0
F. indologenes	57	0	0	10(11)	37	0	0
CDC Ve-1	7	0	0	0	0	0	0
CDC Ve-2	25	1(1)	8	0	0	15(4)	76
P. paucimobilis	28	0	0	0	0	0(2)	7
F. multivorum	8	0	0	6	75	0	0
P. vesicularis	8	0	0	0	0	0	0
P. acidovorans	27	26(3)	100	11(15)	96	27	100
P. alcaligenes	15	0	0	0	0	0	0
P. testosteroni	19	0	0	6	32	2	11
P. diminuta	13	0	0	0	0	0	0

Table 1. (continued)

Organism	No. Tested	Acetamide		Allantoin		Tartrate	
		+(+)[a]	%+	+(+)	%+	+(+)	%+
P. putrefaciens	5	0	0	0	0	0	0
A. odorans	23	22(1)	100	0	0	3	13
A. denitrificans	8	0	0	7	88	0	0
A. faecalis	14	0(3)	21	1	7	3	21
CDC IVc	15	0	0	14(1)	100	12(2)	93
B. bronchiseptica	7	0	0	1(2)	43	0	0
Alcaligenes species	8	0	0	8	100	0	0

[a]Symbols: +, number positive within 2 days; (+), number positive within 3 to 7 days; %+, percent positive after 7 days; w, weak reaction.
[b]UFP, unidentified fluorescent pseudomonads.

(b) Add the amides and salts to the respective aliquots of base medium in the following concentrations: acetamide, 1% (10 g/liter); allantoin, butyramide, formamide, hippurate, nicotinamide, propionate, and tartrate, 0.5% (5 g/liter). Acetamide, propionate, sodium and potassium tartrate are available from Fischer Scientific Company, Fairlawn, NJ; and allantoin, butyramide, formamide, hippuric acid (sodium salt), and nicotinamide are available from Eastman Kodak Company, Rochester, N.Y.

(c) Dispense in 3-ml amounts into 13 × 100 mm screw-cap tubes.

(d) Autoclave at 121°C for 15 minutes, and allow the medium to cool in the slanted position.

(e) *Final pH.* 6.9 ± 0.1.

(f) *Expiration.* 3 months at 4°C.

(g) Controls

(i) Acetamide and allantoin. *Pseudomonas aeruginosa* or *P. acidovorans.*

(ii) *Tartrate. P. acidovorans.*

BETA-GALACTOSIDASE (ONPG) TEST

DESCRIPTION

Many organisms are unable to ferment lactose but possess mechanisms to break down the lactose molecule into one molecule of glucose and one of

galactose. The beta-galactosidase test is based on the hydrolysis on *o*-nitrophenyl-beta-D-galactopyranoside (ONPG) by the enzyme beta-galactosidase, to form *o*-nitrophenyl. The reaction is characterized by the formation of a yellow color in the test medium due to the pigmented *o*-nitrophenyl.

TEST PROCEDURE

1. Place on ONPG disk in a small, capped sterile plastic tube. The disks are available commercially.
2. Prepare a dense emulsion of the test organism in saline and add four drops of the suspension to the disk. Alternatively, place 0.2 ml of saline and an ONPG disk in the tube, and add one loopful of growth taken from a slant or agar plate.
3. Incubate the test overnight at 35°C, and examine for a color change.

INTERPRETATION

1. A positive test is indicated by the appearance of a yellow color in the fluid.
2. A negative test is seen as no color change.

NOTES

1. A large volume of fluid should not be added to the disk to prevent dilution of the color intensity.
2. A broth culture also may be used as the inoculum. However, take care not to confuse the straw color of the broth with a weak reaction.
3. When testing yellow-pigmented organisms such as the flavobacteria, a second tube containing inoculum only should be used to differentiate a true reaction from a pigment effect.
4. Fermentation of lactose depends on two enzymes: a permease and a beta-galactosidase. The permease allows the lactose to enter the bacterial cell, and organisms devoid of permease may be late lactose fermenters, or may fail to ferment lactose altogether. The galactosidase, in contrast to permease, can pass through the cell wall and break down the lactose molecule into glucose and galactose. Hence, an organism may lack permease but possess a galactosidase. Organisms devoid of a galactosidase generally lack a permease also.
5. The nonfermentative bacteria do not possess permeases, but many possess a galactosidase. Some organisms, such as *Acinetobacter anitratus*, however,