



QUALITY CONTROL PROCEDURES

I INTRODUCTION

Acetamide Agar is a medium for the differentiation of nonfermentative gram-negative bacteria, particularly *Pseudomonas aeruginosa*.

II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with the cultures listed below.
 - Using a 0.01 mL calibrated loop, inoculate the slant surfaces using 10⁻¹ dilutions of 18- to 24-h **Trypticase™** Soy Broth cultures.
 - Incubate tubes with loosened caps at 35 ± 2 °C in an aerobic atmosphere.
- Examine tubes after 18–24 h and 4 days for growth and reaction and 7 days for negative reactions.
- Expected Results

Organisms	ATCC®	Deamination Reaction
* <i>Pseudomonas aeruginosa</i>	10145	+
		(purplish-red color within 7 days)
* <i>Stenotrophomonas maltophilia</i>	13637	–
		(no purplish-red color developed within 7 days)

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

- Examine tubes as described under “Product Deterioration.”
- Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
- Incubate uninoculated representative tubes at 20–25 °C and 30–35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Acetamide Agar is used in the differentiation of nonfermentative gram-negative bacteria, particularly *Pseudomonas aeruginosa*.

V SUMMARY AND EXPLANATION

Assimilation studies by Gilardi and others using basal mineral media showed that acetamide was utilized by a wide variety of nonfermenting organisms.^{1,2} However, few organisms are reported to deaminate acetamide.^{3,4} A variety of media formulations have been developed to determine the ability of various nonfermenting gram-negative organisms to deaminate acetamide for purposes of identification.⁵⁻⁸ The formulation of this medium is the one recommended in *Standard Methods for the Examination of Water and Wastewater*.⁹

VI PRINCIPLES OF THE PROCEDURE

The ability to deaminate acetamide (acylamidase activity) has been found to be most actively accomplished by *P. aeruginosa*, *Comamonas (Pseudomonas) acidovorans*, *Achromobacter xylosoxidans* subsp. *xylosoxidans* (*Alcaligenes xylosoxidans*) and *Alcaligenes faecalis (odorans)*.⁸ Deamination of acetamide produces ammonia which increases the pH of the medium causing a corresponding color change from yellow-orange to purplish-red.

VII REAGENTS

Acetamide Agar

Approximate Formula* Per Liter Purified Water

Acetamide	10.0 g	Magnesium Sulfate	0.5 g
Sodium Chloride	5.0 g	Phenol Red	0.012 g
Dipotassium Phosphate	1.39 g	Agar	15.0 g
Monopotassium Phosphate	0.73 g		

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

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, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2–25 °C. Avoid freezing and overheating. 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. Minimize exposure to light.

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

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{10,11} Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Acetamide Agar Slants

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Inoculate the Acetamide Agar slant with a loopful of culture emulsified in **Trypticase** Soy Broth. Incubate inoculated slant at 35 ± 2 °C and observe daily for 4 days and again at 7 days before discarding as negative.

User Quality Control: See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Deamination of the acetamide is indicated by a pronounced purplish-red color of the medium.

Complete identification requires determination of the Gram reaction, cellular morphology, biochemical reactions, etc. Appropriate references may be consulted for additional information.^{10,12}

XI LIMITATIONS OF THE PROCEDURE

Some strains deaminate acetamide slowly and may require as long as 7 days to yield a positive test result. Only about 37% of apyocyanogenic strains of *P. aeruginosa* will produce a positive reaction. Therefore, this test should not be relied upon as a sole criterion for identification.¹⁰

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Acetamide Agar slants are tested for performance characteristics. Using a 0.01 mL calibrated loop, representative samples of the lot are streak-inoculated with **Trypticase** Soy Broth cultures of *Pseudomonas aeruginosa* (ATCC 10145) and *Stenotrophomonas maltophilia* (ATCC 13637) diluted 10⁻¹. Inoculated tubes are incubated at 35 ± 2 °C with loosened caps. Tubes are read for growth and reaction after 18–24 h, and 1, 4, and 7 days. Within 7 days, *P. aeruginosa* exhibits fair to heavy growth with a purplish-red color in the agar slant indicating deamination of the acetamide; *S. maltophilia* exhibits fair to heavy growth with no color change indicating that no deamination of the acetamide occurred.

XIII AVAILABILITY

Cat. No. Description

221828 **BD BBL™** Acetamide Agar Slants, Pkg. of 10 size K tubes

XIV REFERENCES

1. Gilardi, G.L. 1974. Nonfermentative gram-negative bacteria encountered in clinical specimens. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 39:229-242.
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3. Pickett, M.J., and M.M. Pedersen. 1970. Characterization of saccharolytic nonfermentative bacteria associated with man. *Can. J. Microbiol.* 16:351-362.
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Technical Information: In the United States, contact BD Technical Service and Support at 800-638-8663 or www.bd.com/ds.



Becton, Dickinson and Company
7 Loveton Circle
Sparks, MD 21152 USA



Benex Limited
Pottery Road, Dun Laoghaire
Co. Dublin, Ireland

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