QUALITY CONTROL PROCEDURES

I INTRODUCTION

Nutrient Agar is a general-purpose medium for the cultivation of a wide variety of bacterial organisms.

II PERFORMANCE TEST PROCEDURE

1. Liquefy Nutrient Agar in A tubes by heating in boiling water. Cool to 45–50 °C and pour into Petri dishes and allow to firm up for at least 30 min.
2. Streak the plates using 0.01 mL calibrated loops in order to obtain isolated colonies. For tubed slants, inoculate the agar surfaces with a loopful of inoculum. Use 10^{-1} dilutions of 18- to 24-h **Trypticase™** Soy Broth cultures of the organisms listed below.
3. Incubate plates or tubes in an aerobic atmosphere at 35 ± 2 °C. Caps should be loosened on the tubed media.
4. Examine plates or tubes after 18–24 h for amount of growth.
5. Expected Results

Organisms	ATCC™	Recovery
Pseudomonas aeruginosa	10145	Moderate to heavy growth, green pigmentation
**Shigella flexneri*	12022	Moderate to heavy growth
**Staphylococcus aureus*	25923	Moderate to heavy growth, cream to gold colonies

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under “Product Deterioration.”
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. 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

Nutrient Agar is used for the cultivation of bacteria and for the enumeration of organisms in water, sewage, feces and other materials.

V SUMMARY AND EXPLANATION

Early in the 20th century, the American Public Health Association published the formula for a general purpose medium for the growth of a wide variety of nonfastidious microorganisms.[1] This was in recognition of the need for a standardized medium for use in the examination of water and wastewater, dairy products and various foods. This relatively simple formulation has stood the test of time, and with the name of Nutrient Agar, is still specified in current compendia of methods for the microbiological examination of a broad spectrum of materials.[2-5] Additionally, it is used in the laboratory for the cultivation and maintenance of nonfastidious species.

VI PRINCIPLES OF THE PROCEDURE

Nutrient Agar consists of peptone, beef extract and agar. This relatively simple formulation provides the nutrients necessary for the replication of a large number of microorganisms which are not excessively fastidious. The beef extract contains water-soluble substances including carbohydrates, vitamins, organic nitrogen compounds and salts. Peptones are the principle sources of organic nitrogen, particularly amino acids and long-chained peptides. Agar is the solidifying agent.

VII REAGENTS

Nutrient Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin	5.0 g
Beef Extract	3.0 g
Agar	15.0 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.[6,7] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Nutrient agar

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

Test Procedure: Observe aseptic techniques.

Liquefy the agar contained in A tubes, cool to 45–50 °C and pour into Petri dishes. Allow to solidify for at least 30 min. Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. Incubate plates at 35 ± 2 °C for 18 –24 h and 42–48 h, if necessary.

Tubed slants are used primarily for the cultivation and maintenance of pure cultures. They should be inoculated with an inoculating loop and incubated under the same conditions as the plated medium.

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

After incubation, most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Further, growth of each organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas.

Growth from tubes inoculated with pure cultures may be used for biochemical and/or serological testing.

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[6-8]

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Nutrient Agar slants and tubes 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 diluted 10^{-1} of *Pseudomonas aeruginosa* (ATCC 10145), *Shigella flexneri* (ATCC 12022) and *Staphylococcus aureus* (ATCC 25923). Inoculated containers are incubated at 35 ± 2 °C with loosened caps. Containers are read for growth and pigmentation after 18–24 h incubation. All cultures show moderate to heavy growth; colonies of *P. aeruginosa* exhibit green pigmentation; colonies of *S. aureus* are cream to gold.

XIII AVAILABILITY

Cat. No.	Description
220971	**BBL™** Nutrient Agar Slants, Ctn. of 100 size K tubes
298235	**BBL™** Nutrient Agar Slants, Ctn. of 100 size D tubes
220968	**BBL™** Nutrient Agar Deeps (Pour Tubes), 20 mL, Pkg. of 10 size A tubes

XIV REFERENCES

1. American Public Health Association. 1917. Standard methods of water analysis, 3rd ed. American Public Health Association, New York.
2. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
3. Clesceri, L.S., A.E. Greenberg, and A.D. Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
4. Horwitz, W. (ed.). 2000. Official methods of analysis of AOAC International, 17th ed, vol.1. AOAC International, Gaithersburg, MD.
5. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
6. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller and R.H. Yolken (ed.) 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
7. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
8. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.

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