

colorless zones surrounding colonies after the plate is flooded with Gram's iodine. Blue or purple zones surrounding colonies indicate a negative test.

The decomposition of tyrosine can be detected in Quadrant III. A clear halo around a colony is a positive test. Growth without the presence of clear halos or growth with the production of melanin-like pigment is a negative test.

The ability of isolates to decompose xanthine may be detected in Quadrant IV. A clear halo around a colony is a positive test. Growth without the presence of clear halos or growth with the production of a melanin-like pigment is a negative test.

Procedure

Inoculate each sector with a pure culture of the isolate. Use a small sterile spatula to obtain approximately 1 mm of the colony from a pure culture. Using the spatula, cut a small groove through the agar to the bottom of the plate, depositing the inoculum near the bottom of the groove. Alternatively, the tip of a sterile wooden applicator stick can be used to make a well through the agar to the bottom of the plate, depositing the inoculum at the bottom of the well.

Incubate the plates at 30°C in an inverted position (agar side up) under aerobic conditions and observe every 3-4 days for 14-21 days.¹

Expected Results

Examine plates for growth periodically for 14-21 days of incubation.

Examine Quadrant I for the presence of a clear halo in the white opaque medium around the inoculum, which indicates a positive reaction. *N. brasiliensis* decomposes casein and gives a positive reaction. *N. asteroides* shows a negative reaction or no clearing around the inoculum.

To determine starch utilization, flood Quadrant II with Gram's or Lugol's iodine and observe the plate for colorless zones around the inoculum, which indicates a positive reaction, such as that obtained with *S. rimosus*. *N. asteroides* and *N. brasiliensis* give a negative reaction with no clear zones; blue or purple zones surround colonies.

The decomposition of tyrosine in Quadrant III is indicated by clear halos around colonies. There are no clear halos around colonies in a negative test. *N. brasiliensis* decomposes tyrosine, whereas *N. asteroides* does not.

The ability of isolates to decompose xanthine in Quadrant IV is shown by a clear halo around colonies, such as that obtained with *S. rimosus*. The absence of clear halos or the production of a melanin-like pigment indicates a negative test. Both *N. asteroides* and *N. brasiliensis* give a negative reaction.

References

- Land. 1992. In Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
- Koneman, Allen, Janda, Schreckenberger and Winn. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott-Raven Publishers, Philadelphia, Pa.

Availability

BBL™ Nocardia ID QUAD

BS10 CMPH MCM7

Cat. No. 298309 Prepared Plates (QUAD) – Pkg. of 10*

*Store at 2-8°C.

Nutrient Agar

Intended Use

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

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 the 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.

User Quality Control

Identity Specifications

Difco™ Nutrient Agar

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	2.3% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear to slightly opalescent.
Prepared Appearance:	Light amber, very slightly to slightly opalescent.
Reaction of 2.3% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Nutrient Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Enterococcus faecalis</i>	19433	10 ² -10 ³	Good
<i>Escherichia coli</i>	25922	10 ² -10 ³	Good
<i>Pseudomonas aeruginosa</i>	27853	10 ² -10 ³	Good

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 that 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.

Formula

Difco™ Nutrient Agar

Approximate Formula* Per Liter		
Beef Extract	3.0	g
Peptone	5.0	g
Agar	15.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 23 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Liquefy the agar if prepared tubes are used, cool to 45-50°C and pour into Petri dishes. Allow to solidify for at least 30 minutes. Use standard procedures to obtain isolated colonies from specimens. Incubate plates at 35 ± 2°C for 18-24 hours and 42-48 hours, 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.

Expected Results

Examine plates for growth.

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

References

1. American Public Health Association. 1917. Standard methods of water analysis, 3rd ed. American Public Health Association, New York, N.Y.
2. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
3. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
4. Horwitz (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.

Availability

Difco™ Nutrient Agar

	AOAC	BAM	CCAM	COMP	ISO	SMWW	USDA
Cat. No. 212000							
213000							
211665							

BBL™ Nutrient Agar

	AOAC	BAM	CCAM	COMP	ISO	SMWW	USDA
<i>United States and Canada</i>							
Cat. No. 297801							
220968							
220971							

Europe

Cat. No. 256035 Prepared Plates – Pkg. of 20*

Mexico

Cat. No. 257500 Prepared Plates – Pkg. of 10*

*Store at 2-8°C.

Nutrient Agar 1.5%

Intended Use

Nutrient Agar 1.5% is used for cultivating a variety of microorganisms and with the addition of blood or other enrichment can be used for cultivating fastidious microorganisms.

Summary and Explanation

Nutrient Agar 1.5% is a modification of Nutrient Agar.¹ This medium is a slightly alkaline general-purpose medium. Since the medium contains 0.8% sodium chloride, it can be used as a base for enrichment with blood, ascitic fluid or other supplements for cultivating fastidious microorganisms.

Principles of the Procedure

Beef extract and peptone provide the nitrogen, vitamins, amino acids and carbon sources in Nutrient Agar 1.5%. Sodium chloride maintains the osmotic balance so that red blood cells

will not rupture when blood is added as supplement.¹ Agar is the solidifying agent.

Formula

Difco™ Nutrient Agar 1.5%

Approximate Formula* Per Liter		
Beef Extract	3.0	g
Peptone	5.0	g
Sodium Chloride	8.0	g
Agar	15.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 31 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.