NA/NA

Code 5550

Nutrient Agar (**NA**)

USE:

Cultivation of a wide variety of non-fastidious bacteria.

Side 1 & 2: Nutrient Agar (**NA**) (colorless, slightly hazy)

APPLICATION

In the early 1900's, the American Public Health Association (APHA) suggested the formula of Nutrient Agar as a standard culture medium used in water testing.[1] Nutrient Agar continues to be a widely used general purpose medium for growing non-fastidious microorganisms. If required, enrichments can be added to this medium.

Nutrient Agar meets APHA and Association of Official Analytical Chemists (AOAC) standard methods.[2] [3] Nutrient Agar is specified in Standard Methods for the Examination of Water and Wastewater procedures for the examination of food, dairy products, water, and other materials.[4]

PADDLE AGAR

Nutrient Agar (NA) – The nitrogen, carbon, vitamins, and amino acids in Nutrient Agar are provided by enzymatic digest of gelatin and beef extract. Agar and a proprietary polymer are the solidifying agents.

Note: Good growth of nonfastidious organisms (bacteria) on Nutrient Agar will appear as translucent colonies.

CULTURE CONTROLS

10-300 inoculum (CFU)

	NA Agar
Bacillus subtilis	GROWTH
Escherichia coli	GROWTH
Aspergillus niger	GROWTH
Saccharomyces cerevesiae	GROWTH

[1] American Public Health Association. 1917. Standard methods of water analysis, 3rd ed. American Public Health Association, Washington, D.C.
[2] Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.). 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
[3] Marshall, R. T. (ed.). 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
[4] Vanderzant, C., and D. F. Splittstoesser (eds.). 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

STORAGE / EXPIRATION

Store tightly sealed BioPaddles® in a cool, dry location. Shield from direct sunlight. Store BioPaddles® at room temperature (65 - 77°F/18 - 25°C). Avoid sudden temperature changes. Temperature fluctuations may result in condensation settling at the bottom of the vial. This will not affect the culture properties but could reduce the shelf-life or cause the agar to separate from the plastic paddle support. Do not refrigerate or store at temperatures above 80°F/27°C. Refrigeration may result in water condensation. Avoid freezing. Freezing can promote excess water loss and variation in media surface due to crystal formation. If freezing occurs, wrap BioPaddle in vial in thick towel and thaw at room temperature for 3-6 hours.

Refer to Best Before End date (See: BBE stamped on vial). Discard if paddle agar appears oxidized and darker than the expected color or if contaminants appear. The expiration date is based on medium in an intact container that is stored as directed.

SAMPLING

Liquids: Twist to remove paddle from vial. Fill vial to 40 mL fill line with the liquid to be sampled. The 40 mL volume can be used to calculate Total Viable Count (TVC) and/or Total Colony Count (TCC). Replace paddle. Allow a contact time of 15 seconds. Remove the paddle. Empty the vial. Replace the paddle in the vial.

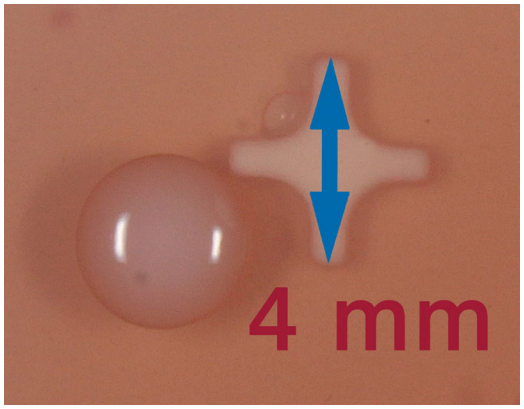
Surfaces: Recovery rate is about 50%. Twist paddle to remove from vial. To ensure an accurate recovery, touch the paddle surface (10 cm^2) to the test surface twice to cover a 20 cm^2 area (2 X 10 cm^2). Allow 15 second contact time. Replace paddle in vial.

INCUBATION

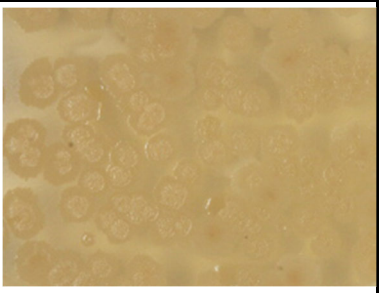
Temperature	Minimum Period	Optimal Period
35°C	18 hours	24 hours
20-25°C	5 days	7 days

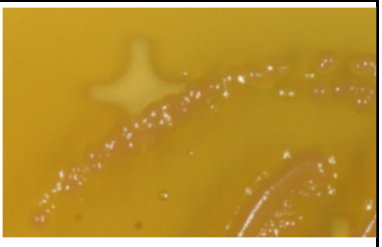
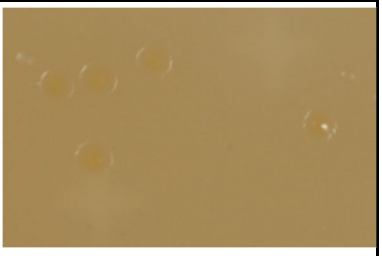
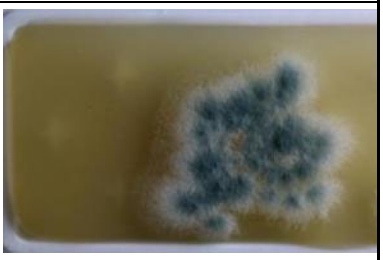
COLONY MEASURING

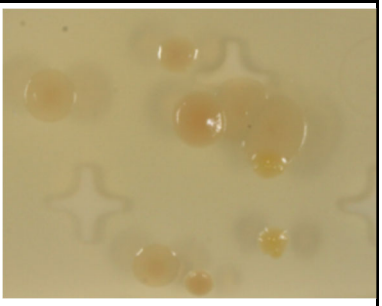
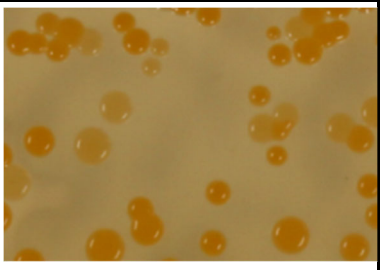
Each BioPaddles® paddle has molded media attachment points that are 4mm in length (point-to-point). This feature provides a useful guidepost to estimating nearby colony size.



IDENTIFICATION

ORGANISM		NA		
ORGANISM	**PHYSIOLOGY** ◆ Precision Test Strip Available	**GROWTH**	**COLONY**	**IMAGE**
Aspergillus niger	• Catalase (+) • Ascomycete	+++	• Granular • Jet black conidia w/ yellow/gray hyphae	
Bacillus spp.	• Lactose (-) • Indole (-) ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Urease () ◆ • Gram (+) Rod • 25 - 30°C	+++	• Translucent to dull, off-white; opaque • Smooth to rough • irregular/dendroid margins to spreading • 2-4mm	
Candida albicans	• Catalase (+) • Ascomycete	+++	• Cream • Convex • Glossy • Entire • 1-2mm	

ORGANISM		NA		
ORGANISM	**PHYSIOLOGY** ◆ Precision Test Strip Available	**GROWTH**	**COLONY**	**IMAGE**
Escherichia coli	• Lactose (+) • Indole (+) ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (-) Rod) • 35 - 37°C	+++	• Translucent; may be dull, off-white, opaque • Convex • Glossy • Entire • 0.5 - 1.0mm	
Enterobacter aerogenes	• Lactose (+) • Indole (-) ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (-) Rod	+++	• Yellow, translucent • Convex • Glossy • Entire • 1-2mm	
Lactobacillus delbrueckii	• Lactose (+) • Indole (-) ◆ • Oxidase (+) ◆ • Catalase (-) ◆ • Urease (-) ◆ • Gram (+) Rod • 40-44°C	+++	• Transparent/Gray • Rough; shiny • Convex, umbonate • 2 - 4mm	
Penicillium chrysogenum	• Catalase (+) • Ascomycete	+++	• Granular, velvet-like/powdery, flat • Initially white, then various shades of green blue-green or yellow-green pigment • 3-9+cm	

ORGANISM		NA		
ORGANISM	**PHYSIOLOGY** ◆ Precision Test Strip Available	**GROWTH**	**COLONY**	**IMAGE**
Pseudomomas fluorescens	• Lactose (-) • Indole (-) ◆ • Oxidase (+) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (-) Rod • Fluoresces blue-green under long-wave UV light (400-nm) • 25-30°C	+++	• Translucent to amber • Irregular; Spreading to confluent • Clear to grayish with dark centers (translucent edges) • Diffusible green-blue pigment • 2-4mm	
Staphylococcus aureus	• Lactose (-) • Indole (-) ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (+) Sphere • 35 - 37°C	+++	• Yellowish-gold/Opaque • Convex (butyrous) • Glossy • Entire • 2 - 4mm	

+++ = very rich, luxurious growth expected
++ = grows
+ = grows slightly
+/- = may grow; may be inhibited

DISPOSAL

Twist to remove paddle from vial. Fill vial to 40 mL fill line with 1:9 dilution of household bleach (5.25% sodium hypochlorite). Replace paddle in vial. Allow 15 minute contact time. Remove paddle. Discard bleach solution. Replace paddle in vial and dispose. Alternatively, loosen cap and microwave for 30 seconds, autoclave, or incinerate.

GLOSSARY:

Catalase Test — Catalase enzyme will react with hydrogen peroxide to produce oxygen if the bacteria is catalase positive.

Lactose Test — Lactose positive bacteria can ferment available lactose in the agar producing an acid which lowers the pH. Lactose negative bacteria are non-fermenting.

Indole Test — Biochemical test to determine the ability of an organism to split indole from the amino acid tryptophan. *P. vulgaris* is indole positive while *P. mirabilis* is indole negative.

Oxidase Test	Oxidase positive bacteria contain cytochrome c oxidase which will turn an indicator dark blue. In contact with oxidase negative bacteria, the indicator will remain colorless.
Urease Test	Bacteria containing urease will hydrolyze urea to ammonia and carbon dioxide causing an alkaline environment which changes the color of a pH indicator from yellow to fuchsia.
β-D-Glucoronidase Reaction	The presence of *E. coli* is determined when both β-D-Glucoronidase and Indole are positive, and the organism is gram negative.
Gram Staining	A method for differentiating bacteria into two groups – gram positive and gram negative – based on the chemical and physical properties of their cell walls. Often the first step in identifying bacteria.