

Expected Results

Examine plates for growth and hemolytic reactions after 18-24 and 40-48 hours of incubation. Four different types of hemolysis on blood agar media can be described:⁷

- Alpha (α)-hemolysis is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony, causing a greenish discolorization of the medium.
- Beta (β)-hemolysis is the lysis of red blood cells, resulting in a clear zone surrounding the colony.
- Gamma (γ)-hemolysis indicates no hemolysis. No destruction of red blood cells occurs, and there is no change in the medium.
- Alpha-prime (α')-hemolysis is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

Limitations of the Procedure

- Azide Blood Agar Base is intended for selective use and should be inoculated in parallel with nonselective media.
- Hemolytic patterns of streptococci grown on Azide Blood Agar Base are somewhat different than those observed on ordinary blood agar. Sodium azide enhances hemolysis. Alpha and beta zones may be extended.⁴
- Hemolytic patterns may vary with the source of animal blood or base medium used.⁶

Azide Dextrose Broth

Intended Use

Azide Dextrose Broth is used for cultivating streptococci in water and wastewater.

Summary and Explanation

The formula for Azide Dextrose Broth originated with Rothe at the Illinois State Health Department.¹ In a comparative study, Mallmann and Seligmann² investigated the detection of streptococci in water and wastewater using Azide Dextrose Broth. Their work supported use of the medium in determining the presence of streptococci in water, wastewater, shellfish and other materials. Azide Dextrose Broth has also been used for primary isolation of streptococci from foodstuffs^{3,4} and other specimens of sanitary significance as an indication of fecal contamination.

Azide Dextrose Broth is specified for use in the presumptive test of water and wastewater for fecal streptococci by the Multiple-Tube Technique.⁵

Principles of the Procedure

Azide Dextrose Broth contains beef extract and peptones as sources of carbon, nitrogen, vitamins and minerals. Dextrose is a fermentable carbohydrate. Sodium chloride maintains the osmotic balance of the medium. Sodium azide inhibits cytochrome oxidase in gram-negative bacteria.

References

- Edwards. 1933. J. Comp. Pathol. Therap. 46:211.
- Snyder and Lichstein. 1940. J. Infect. Dis. 67:113.
- Lichstein and Snyder. 1941. J. Bacteriol. 42:653.
- Packer. 1943. J. Infect. Dis. 67:113.
- Mallmann, Botwright and Churchill. 1943. J. Bacteriol. 46:343.
- Ruoff, Whiley and Beighton. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
- Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Azide Blood Agar Base

Cat. No. 240920 Dehydrated – 500 g

Group D streptococci grow in the presence of azide, ferment glucose and cause turbidity.

Formula

Difco™ Azide Dextrose Broth

Approximate Formula* Per Liter

Beef Extract	4.5	g
Pancreatic Digest of Casein	7.5	g
Proteose Peptone No. 3	7.5	g
Dextrose	7.5	g
Sodium Chloride	7.5	g
Sodium Azide	0.2	g

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

Directions for Preparation from Dehydrated Product

- Dissolve 34.7 g of the powder in 1 L of purified water for the preparation of single-strength broth for inoculation of samples of 10 mL or smaller. Use 69.4 g for 1 L of double-strength broth for samples larger than 10 mL.
- Autoclave at 121°C for 15 minutes.
- Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ Azide Dextrose Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.47% (single strength) solution, soluble in purified water. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to very slightly opalescent.
Reaction of 3.47% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Azide Dextrose Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

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



Procedure⁵

1. Inoculate a series of Azide Dextrose Broth tubes with appropriately graduated quantities of sample. Use sample quantities of 10 mL or less. Use double-strength broth for 10 mL inocula. Consult an appropriate reference for suggested sample sizes.⁵
2. Incubate inoculated tubes at 35 ± 2°C for 20-48 hours.
3. Examine each tube for turbidity at the end of 24 ± 2 hours. If no turbidity is evident, reincubate and read again at the end of 48 ± 3 hours.

Expected Results

A positive test is indicated by turbidity (cloudiness) in the broth. A negative test remains clear.

All Azide Dextrose Broth tubes showing turbidity after 24- or 48-hours of incubation must be subjected to the Confirmed Test Procedure. Consult appropriate references for details of the Confirmed Test Procedure⁵ and further identification of *Enterococcus*.^{5,6}

Limitations of the Procedure

1. Azide Dextrose Broth is used to detect presumptive evidence of fecal contamination. Further biochemical testing must be done for confirmation.
2. For inoculum sizes of 10 mL or larger, use double strength medium to prevent dilution of ingredients.^{5,6}

References

1. Rothe. 1948. Illinois State Health Department.
2. Mallmann and Seligmann. 1950. Am. J. Public Health 40:286.
3. Larkin, Litsky and Fuller. 1955. Appl. Microbiol. 3:98.
4. Splittstoesser, Wright and Hucker. 1961. Appl. Microbiol. 9:303.
5. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
6. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ Azide Dextrose Broth

SMWW

Cat. No. 238710 Dehydrated – 500 g