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 Seligmann[2] 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.

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.*

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
Enterococcus faecalis	19433	10^2-10^3	Good
Escherichia coli	25922	3 10^2-10^3	Inhibition

Uninoculated Tube

Enterococcus faecalis ATCC™ 29212

Directions for Preparation from Dehydrated Product

1. 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.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure[5]

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 Procedure[5] 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. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. 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

EPA SMWW

Cat. No. 238710 Dehydrated – 500 g