

buffers are incorporated to maintain the pH of the medium. Sodium citrate and sodium desoxycholate are added to inhibit gram-positive and some gram-negative bacteria.

Proteus, *Pseudomonas* and coliforms do not overgrow *Salmonella* and *Shigella* in GN Broth during the first 6 hours of incubation.

Formulae

Difco™ GN Broth, Hajna

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|-----------------------------------|--------|
| Approximate Formula* Per Liter | |
| Pancreatic Digest of Casein | 12.0 g |
| Proteose Peptone No. 3 | 8.0 g |
| Dextrose | 1.0 g |
| D-Mannitol | 2.0 g |
| Sodium Citrate | 5.0 g |
| Sodium Desoxycholate | 0.5 g |
| Dipotassium Phosphate | 4.0 g |
| Monopotassium Phosphate | 1.5 g |
| Sodium Chloride | 5.0 g |

BBL™ GN Broth

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|--------------------------------------|--------|
| Approximate Formula* Per Liter | |
| Pancreatic Digest of Casein | 10.0 g |
| Peptic Digest of Animal Tissue | 10.0 g |
| Dextrose | 1.0 g |
| D-Mannitol | 2.0 g |
| Sodium Citrate | 5.0 g |
| Sodium Desoxycholate | 0.5 g |
| Dipotassium Phosphate | 4.0 g |
| Monopotassium Phosphate | 1.5 g |
| Sodium Chloride | 5.0 g |

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

Directions for Preparation from Dehydrated Product

1. Suspend 39 g of the powder in 1 L of purified water. Mix thoroughly.
2. Dispense and autoclave at 121°C for 15 minutes.
3. Alternatively, the broth may be steamed for 30 minutes at 100°C.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate the broth as soon as possible after the specimen arrives at the laboratory. Swab specimens may be inserted

directly into the broth. For stool specimens, use 1 g of feces or 1 mL of liquid stool per tube. Consult appropriate references for information about the processing and inoculation of other clinical specimens or food samples.⁶⁻⁹

Incubate the tubes with loosened caps at 35 ± 2°C and subculture onto selective and differential media after 6-8 hours of incubation and again after 18-24 hours of incubation.¹⁰

Expected Results

Growth in broth media is indicated by turbidity compared to an uninoculated control. Subculture onto appropriate selective and differential media to isolate pathogens for identification.

Limitation of the Procedure

Enrichment broths should not be used as the sole isolation medium. They are to be used in conjunction with selective and nonselective plating media to increase the probability of isolating pathogens, especially when they may be present in small numbers. Consult references for detailed information and recommended procedures.⁶⁻⁹

References

1. Hajna. 1955. Public Health Lab. 13:59.
2. Hajna. 1955. Public Health Lab. 13:83.
3. Croft and Miller. 1956. Am. J. Clin. Pathol. 26:411.
4. Taylor and Schelhart. 1967. Am. J. Clin. Pathol. 48:356.
5. Taylor and Schelhart. 1968. Appl. Microbiol. 16:1383.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
7. Farmer. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
8. Forbes, Sahm and Weissfeld. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis, Mo.
9. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
10. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ GN Broth, Hajna

BS10 CMPH COMPF MCM7 USDA

Cat. No. 248610 Dehydrated – 500 g

BBL™ GN Broth

BS10 CMPH COMPF MCM7 USDA

Cat. No. 211279 Dehydrated – 500 g

221729 Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10*

221730 Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100*

*Store at 2-8°C.

Gelatin

Intended Use

Gelatin is used in preparing microbiological culture media.

Summary and Explanation

Gelatin is a protein of uniform molecular constitution derived chiefly by the hydrolysis of collagen.¹ Collagens are a class of albuminoids found abundantly in bones, skin, tendon, cartilage and similar animal tissues.¹

Koch¹ introduced gelatin into bacteriology when he invented the gelatin tube method in 1875 and the plate method in 1881.

This innovation, a solid culture method, became the foundation for investigation of the propagation of bacteria.¹ However, gelatin-based media were soon replaced by media containing agar as the solidifying agent.

Gelatin is used in culture media for determining gelatinolysis (elaboration of gelatinases) by bacteria. Levine and Carpenter² and Levine and Shaw³ employed gelatin media in their studies of gelatin liquefaction. Garner and Tillett⁴ used culture media prepared with gelatin to study the fibrinolytic activity of hemolytic streptococci.

User Quality Control

Identity Specifications

Difco™ Gelatin

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|------------------------|--|
| Dehydrated Appearance: | Light beige, free-flowing, homogeneous. |
| Solution: | 12% solution, soluble in purified water upon slight heating in a 50-55°C water bath. Solution is light amber, clear to slightly opalescent, may have a slight precipitate. |
| Prepared Gel: | Very light amber, clear to slightly opalescent, may have a slight precipitate. |

Cultural Response

Difco™ Gelatin

Prepare a 12% Gelatin solution in 0.8% Nutrient Broth. Dispense into tubes and autoclave. Inoculate and incubate at $35 \pm 2^\circ\text{C}$ under appropriate atmospheric conditions for 18-48 hours or for up to 2 weeks for the gelatinase test. To read gelatinase, refrigerate until well-chilled and compare to uninoculated tubes. Tubes positive for gelatinase will remain liquid.

| ORGANISM | ATCC™ | INOCULUM CFU | RECOVERY | GELATINASE |
|-------------------------------|-------|-----------------|----------|------------|
| <i>Bacillus subtilis</i> | 6633 | 10^2 - 10^3 | Good | + |
| <i>Clostridium sporogenes</i> | 11437 | 10^2 - 10^3 | Good | + |
| <i>Escherichia coli</i> | 25922 | 10^2 - 10^3 | Good | - |



Gelatin is a high grade gelatin in granular form which may be used as a solidifying agent or may be incorporated into culture media for various uses. Gelatin is used in Nutrient Gelatin, Motility GI Medium, Stock Culture Agar and Dextrose Starch Agar. A 0.4% gelatin medium is used in the presumptive differentiation of *Nocardia brasiliensis* from *N. asteroides* (see Nocardia Differentiation Media). Media containing gelatin are specified in standard methods^{5,6} for multiple applications.

Principles of the Procedure

The melting point of a 12% concentration of gelatin is between 28 and 30°C, which allows it to be used as a solidifying agent. Certain microorganisms elaborate gelatinolytic enzymes (gelatinases) which hydrolyze gelatin, causing liquefaction of a solidified medium or preventing the gelation of a medium containing gelatin. Gelatin is also used as a source of nitrogen and amino acids.

Procedure

See appropriate references for specific procedures using gelatin.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Gershenfeld and Tice. 1941. J. Bacteriol. 41:645.
2. Levine and Carpenter. 1923. J. Bacteriol. 8:297.
3. Levine and Shaw. 1924. J. Bacteriol. 9:225.
4. Garner and Tillett. 1934. J. Exp. Med. 60:255.
5. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
6. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Gelatin

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| Cat. No. | 214340 | Dehydrated – 500 g |
| | 214320 | Dehydrated – 10 kg |