

2. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of the pathogenic *Brucella* spp. and for experimental animal studies.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of **Bacto Casitone**, **Trypticase Peptone**, **Bacto Tryptone** and **BiTek Tryptone** in the formula of the medium being prepared. Add appropriate product as required.

Procedure

See appropriate references for specific procedures using **Bacto Casitone**, **Trypticase Peptone**, **Bacto Tryptone** and **BiTek Tryptone**.

Expected Results

Refer to appropriate references and procedures for results.

References

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2. Horowitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed. AOAC International, Gaithersburg, Md.
3. United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.
4. Takahashi and Yamada. 2000. J. Bacteriol. 182:4704.
5. Nagel, Oostra, Tramper and Rinzema. 1999. Process Biochem. 35: 69.
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. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.

8. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington D.C.
9. Marshall (ed.). 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington D.C.
10. U.S. Environmental Protection Agency. 2000. Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli*. EPA-821/R-97/004. Office of Water, Washington, D.C.
11. U.S. Department of Agriculture. 1998. Microbiology laboratory guidebook, 3rd ed. Food Safety and Inspection Service, USDA, Washington, D.C.
12. Council of Europe. 2002. European pharmacopoeia, 4th ed. Council of Europe, Strasbourg, France.
13. Sivakesavs, Chen, Hackett, Huang, Lam, Lam, Siu, Wong and Wong. 1999. Process Biochem. 34:893.
14. Wen and Chen. 2001. Enzyme Microbia Technol. 29:341.
15. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 1999. Biosafety in microbiological and biomedical laboratories, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.

Availability

Bacto™ Casitone

COMPF SMD SMWW USDA

Cat. No. 225930 Dehydrated – 500 g
225910 Dehydrated – 10 kg

BBL™ Trypticase™ Peptone

AOAC BAM COMPF EP EPA SMD SMWW USDA USP

Cat. No. 211921 Dehydrated – 454 g
211922 Dehydrated – 5 lb (2.3 kg)
211923 Dehydrated – 25 lb (11.3 kg)

Bacto™ Tryptone

AOAC BAM COMPF EP EPA SMD SMWW USDA USP

Cat. No. 211705 Dehydrated – 500 g
211699 Dehydrated – 2 kg

BiTek™ Tryptone

Cat. No. 251420 Dehydrated – 10 kg

Casman Agar Base

Intended Use

Casman Agar Base is used for the cultivation of fastidious pathogenic organisms, such as *Haemophilus influenzae* and *Neisseria gonorrhoeae*, from clinical specimens.

Summary and Explanation

Members of the genus *Haemophilus* are fastidious microorganisms that require the addition of X and/or V growth factors for *in vitro* cultivation.¹ *Neisseria* are also fastidious microorganisms with complex growth requirements.²

In 1947, Casman described a blood-enriched medium prepared without an infusion of fresh meat for cultivation of *Haemophilus* and gonococci.¹ The medium was developed to replace previous formulations that required time-consuming preparations of fresh and heated blood and fresh meat infusion to supply the nutrients necessary for the growth of these fastidious organisms.^{2,3}

Casman found that nicotinamide interfered with the activity of an enzyme in blood that inactivates V factor (NAD). Using unheated human blood, he found that amount of nicotinamide required for good growth of *H. influenzae* was inhibitory to gonococci.² Therefore, he reduced the nicotinamide to a level that allowed good growth of gonococci.

User Quality Control

Identity Specifications

BBL™ Casman Agar Base

| | |
|------------------------------------|--|
| Dehydrated Appearance: | Fine, homogeneous, free of extraneous material. |
| Solution: | 4.3% solution, soluble in purified water upon boiling. Solution is medium to dark, yellow to tan, hazy to cloudy, with a moderate to large amount of cream flocculation. |
| Prepared Appearance: | Medium to dark, yellow to tan, hazy to cloudy, with a moderate to large amount of cream flocculation. |
| Reaction of 4.3% Solution at 25°C: | pH 7.3 ± 0.2 |

Cultural Response

BBL™ Casman Agar Base

Prepare the medium per label directions. Inoculate and incubate for 42-48 hours at 35 ± 2°C, aerobically for *L. monocytogenes* and with 3-5% CO₂ for all other organisms.

| ORGANISM | ATCC™ | INOCULUM CFU | RECOVERY | HEMOLYSIS |
|-------------------------------------|-------|----------------------------------|----------|-----------|
| <i>Haemophilus influenzae</i> | 10211 | 10 ² -10 ³ | Good | N/A |
| <i>Haemophilus parahaemolyticus</i> | 10014 | 10 ² -10 ³ | Good | Beta |
| <i>Listeria monocytogenes</i> | 19115 | 10 ² -10 ³ | Good | Weak beta |
| <i>Neisseria gonorrhoeae</i> | 43070 | 10 ² -10 ³ | Good | N/A |
| <i>Streptococcus pyogenes</i> | 19615 | 10 ² -10 ³ | Good | Beta |

To improve the recovery of *H. influenzae* on this medium, horse or rabbit blood should be used instead of human blood, since they contain less NADase.⁴

Principles of the Procedure

Casman Agar Base is a nonselective, peptone-based medium. The peptones and beef extract provide amino acids and other complex nitrogenous nutrients. Yeast extract is a source of the B-complex vitamins.

Supplementing Casman Agar Base with blood supplies the growth factors required by *H. influenzae* – hemin, or X factor, and nicotinamide adenine dinucleotide (NAD), or V factor. Horse and rabbit bloods are preferred by some laboratories because they are relatively free of NADase, an enzyme that destroys the V factor. The addition of lysed blood stimulates the growth of some strains of *N. gonorrhoeae*. Nicotinamide is incorporated into the medium to inhibit the nucleotidase of erythrocytes that destroys the V factor.

Cornstarch is incorporated to prevent fatty acids from inhibiting the growth of *N. gonorrhoeae* and to facilitate β -hemolytic reactions by neutralizing the inhibitory action of dextrose. A small amount of dextrose is added to enhance the growth of pathogenic cocci.

Formula

BBL™ Casman Agar Base

Approximate Formula* Per Liter

| | | |
|--------------------------------------|------|---|
| Pancreatic Digest of Casein | 11.5 | g |
| Peptic Digest of Animal Tissue | 5.0 | g |
| Yeast Extract | 3.5 | g |
| Beef Extract | 3.0 | g |
| Nicotinamide | 0.05 | g |
| <i>p</i> -Aminobenzoic Acid | 0.05 | g |
| Dextrose | 0.5 | g |
| Cornstarch | 1.0 | g |
| Sodium Chloride | 5.0 | g |
| Agar | 13.5 | g |

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

Directions for Preparation from Dehydrated Product

1. Suspend 43 g of 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. Cool to 45-50°C and add 5% sterile blood and 0.15% blood solution, made by lysing 1 part of blood with 3 parts of water. Alternatively, add 5% partially lysed blood.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion on the isolation and identification of *Neisseria* and *Haemophilus*, consult appropriate references.^{5,6}

Expected Results

H. influenzae produces colorless to gray, transparent, moist colonies with a characteristic “mousy” odor. *N. gonorrhoeae* produces small, translucent, raised, moist, colorless to grayish-white colonies.

Gram staining, biochemical tests and serological procedures should be performed to confirm findings.

References

1. Casman. 1947. Am. J. Clin. Pathol. 17:281.
2. Casman. 1942. J. Bacteriol. 43:33.
3. Casman. 1947. J. Bacteriol. 53:561.
4. Krumweide and Kuttner. 1938. J. Exp. Med. 67:429.
5. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
6. Murray, Baron, Pfaller, Tenover and Tenover (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Casman Agar Base

Cat. No. 211106 Dehydrated – 500 g

Cetrimide Agar Base • Pseudosel™ Agar

Intended Use

This medium conforms with specifications of *The United States Pharmacopeia (USP)*.

Cetrimide (Pseudosel) Agar is used for the selective isolation and identification of *Pseudomonas aeruginosa*.

Summary and Explanation

King et al. developed Medium A (Tech Agar) for the enhancement of pyocyanin production by *Pseudomonas*.¹ Cetrimide (Pseudosel) Agar has the formula for Tech Agar but is modified by the addition of cetrimide for the selective inhibition of organisms other than that *P. aeruginosa*.²

In 1951, Lowbury described the use of 0.1% cetrimide in a selective medium for *P. aeruginosa*.² Because of the increased purity of the inhibitory agent, the concentration was later

reduced, as reported by Lowbury and Collins in 1955.³ Brown and Lowbury employed incubation at 37°C with examination after 18 and 42 hours of incubation.⁴

Strains of *P. aeruginosa* are identified from specimens by their production of pyocyanin, a blue, water-soluble, nonfluorescent, phenazine pigment in addition to their colonial morphology⁵ and the characteristic grapelike odor of aminoacetophenone.⁶ *P. aeruginosa* is the only species of *Pseudomonas* or gram-negative rod known to excrete pyocyanin. Cetrimide (Pseudosel) Agar, therefore, is a valuable culture medium in the identification of this organism.

Cetrimide (Pseudosel) Agar is widely recommended for use in the examination of cosmetics,⁷ pharmaceuticals⁸ and clinical specimens^{5,9} for the presence of *P. aeruginosa*, as well as for evaluating the efficacy of disinfectants against this organism.¹⁰