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% CO_2 for all other organisms.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HEMOLYSIS
Haemophilus influenzae	10211	10^2-10^3	Good	N/A
Haemophilus parahaemolyticus	10014	10^2-10^3	Good	Beta
Listeria monocytogenes	19115	10^2-10^3	Good	Weak beta
Neisseria gonorrhoeae	43070	10^2-10^3	Good	N/A
Streptococcus pyogenes	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
p-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 and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
6. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Casman Agar Base

Cat. No. 211106 Dehydrated – 500 g