Casman Agar

M201

Casman Agar with blood is used for isolation of fastidious microorganisms from clinical specimens under reduced oxygen tension.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Tryptose	10.000
Beef extract	3.000
Dextrose	0.500
Corn starch	1.000
Sodium chloride	5.000
Nicotinamide	0.050
p-Amino benzoic acid (PABA)	0.050
Agar	14.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 43.6 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add 0.15% v/v sterile water lysed blood (water: blood:: 3:1) of 5% sterile blood.Alternatively add 5% partially lysed blood. Mix well and dispense as desired.

Principle And Interpretation

Fastidious microorganisms such as *Haemophilus* and *Neisseria* require the addition of X and V- growth factors for in vitro cultivation (1). Casman (1, 2, 3) described a blood-enriched medium for cultivation of *Haemophilus* and gonococci (1). The medium was developed to replace the previously described formulations that required time-consuming preparations using fresh and heated blood and meat infusion to supply the essential nutrients for growth of these fastidious organisms (2, 3). Blood supplies factor-X (hemin) and factor-V (Nicotinamide Adenine Dinucleotide), which is required for growth of *Haemophilus influenzae* . Sheep blood lacks factor-V due to NADase, an enzyme that destroys factor- V (4). Horse and rabbit blood supplies both the factor X and factor V, and are relatively free of NADase activity, therefore it is preferred over sheep blood. Nicotinamide is added to medium to inhibit nucleotidase of erythrocytes that may destroy factor V.

Proteose peptone, tryptose and beef extract provide amino acids and other complex nitrogenous nutrients. Dextrose improves growth of pathogenic cocci. Corn starch prevents fatty acids from inhibiting the growth of *Neisseria gonorrhoeae* , without interfering with haemolytic reaction. Corn starch also neutralizes the inhibitory action of dextrose. Inoculate the medium as soon as the specimen arrives at the laboratory. After incubation *H. influenzae* produces colourless to grey colonies with a characteristic mousy odour while *N. gonorrhoeae* produces small colourless to greyish-white colonies.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.4% Agar gel.

Colour and Clarity of prepared medium

Basal medium: Yellow coloured clear to slightly opalescent gel. After addition of 5%w/v sterile defibrinated blood : Cherry red coloured After addition of 5%w/v sterile defibrinated blood: opaque gel forms in Petri plates.

Reaction

Reaction of 4.36% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
M201: Cultural characteristics observed with added water-lysed blood, after an incubation at 35-37°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Cultural Response				
Haemophilus influenzae ATCC 35056	50-100	good	50-70%	none
Neisseria meningitidis ATCC 13090	50-100	luxuriant	>=70%	none
Streptococcus mitis ATCC 9811	50-100	luxuriant	>=70%	beta
Streptococcus pneumoniae ATCC 6303	50-100	luxuriant	>=70%	alpha
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	>=70%	beta

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
1. Casman, 1947, Am. J. Clin. Pathol., 17:281.
2. Casman, 1942, J. Bact., 43:33.
3. Casman, 1947, J. Bact., 53:561.
4. Krunveide and Kuttner, 1938, J. Exp. Med., 67:429.

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