Casman Agar

M201

Intended Use:

Recommended for isolation of fastidious microorganisms from clinical specimens under reduced oxygen tension.

Composition**

Ingredients	g/ L
Proteose peptone	10.000
Tryptose	10.000
HM peptone B	3.000
Dextrose (Glucose)	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 purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°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 HM peptone B 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.

Type of specimen

Clinical samples : vaginal swabs, rectal swabs, etc.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further biochemical and serological tests must be carried out for further identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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
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
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 between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

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.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W.(2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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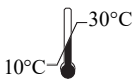
IVD ***In vitro*** **diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE Marking

Do not use if package is damaged

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