

# TM 513 – CASMAN AGAR

#### **INTENDED USE**

For isolation of fastidious bacteria from clinical samples under reduced oxygen tension.

#### PRODUCT SUMMARY AND EXPLANATION

Fastidious microorganisms such as *Haemophilus* and *Neisseria* require the addition of X and V- growth factors for in vitro cultivation. Casman described a blood-enriched medium for cultivation of *Haemophilus* and gonococci. 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. 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. 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.

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 greyishwhite colonies.

### **COMPOSITION**

Ingredients	Gms / Ltr
Proteose peptone	10.000
Tryptose	10.000
Beef extract	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

## **PRINCIPLE**

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.

#### **INSTRUCTION FOR USE**

- Dissolve 43.6 grams in 1000 ml purified / distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi 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.

### **QUALITY CONTROL SPECIFICATIONS**















**Appearance of Powder** : Cream to yellow homogeneous free flowing powder.

Appearance 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.

pH (at 25°C) : 7.3±0.2

### **INTERPRETATION**

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Haemolysis	Incubation Temperature	Incubation Period
Haemophilus influenzae	35056	50-100	Good	40-50%	None	35-37°C	40-48 Hours
Neisseria meningitidis	13090	50-100	Luxuriant	>=70%	None	35-37°C	40-48 Hours
Streptococcus mitis	9811	50-100	Luxuriant	>=70%	Beta	35-37°C	40-48 Hours
Streptococcus pneumoniae	6303	50-100	Luxuriant	>=70%	Alpha	35-37°C	40-48 Hours
Streptococcus pyogenes	19615	50-100	Luxuriant	>=70%	Beta	35-37°C	40-48 Hours

### **PACKAGING:**

In pack size of 500 gm bottles.

## **STORAGE**

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

**Product Deterioration:** Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

# **DISPOSAL**

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

# **REFERENCES**

- 1. Casman, 1947, Am. J. Clin. Pathol., 17:281.
- 2. Casman, 1942, J. Bact., 43:33.
- 3. Casman, 1947, J. Bact., 53:561.
- 4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
- 5. 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.







































**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices. \*For Lab Use Only Revision: 08 Nov., 2019







