

**Glucose Cysteine Agar Base w/ Thiamine****M433**

Glucose Cysteine Agar Base w/ Thiamine with added blood or haemoglobin or hemin is used for cultivation and enumeration of *Francisella tularensis* (*Pasteurella tularensis*) .

**Composition\*\***

Ingredients	Gms / Litre
Meat peptone	3.000
Papaic digest of soyabean meal	10.000
Sodium chloride	5.000
Cysteine hydrochloride	1.000
Dextrose	25.000
Thiamine	0.0005
Agar	14.000
Final pH ( at 25°C)	6.9±0.2

\*\*Formula adjusted, standardized to suit performance parameters

**Directions**

Suspend 58 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 45-50°C and aseptically add sterile packed erythrocytes at a final concentration of 2% or 4-5% defibrinated sheep/rabbit blood. Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

*Francisella tularensis* , a gram-negative aerobic bacillus, is the etiological agent of tularemia, which is primarily a disease of wild animals that is perpetuated in nature by ectoparasites, contaminated environment, cannibalism and acute or chronic carriers. Biting and blood sucking insects serve as vectors (1). *Francisella* (formerly known as *Pasteurella* ) cannot be cultured on ordinary medium but require a complex medium containing blood or tissue extracts, thiamine and cysteine (2, 3). Glucose Cysteine Agar Base w/ Thiamine when supplemented with blood / haemoglobin is recommended for cultivation and enumeration of *F.tularensis* ( *Pasteurella tularensis* )(4).

Meat peptone and papaic digest of soyabean meal provide essential growth nutrients. Dextrose serves as an easily metabolisable carbohydrate source while sodium chloride maintains the osmotic balance. Thiamine and cysteine hydrochloride serves as growth factor promoters required for culturing *Pasteurella* . Minute droplet like colonies develops in 48 hours.

**Quality Control****Appearance**

Cream to yellow homogeneous free flowing powder

**Gelling**

Firm, comparable with 1.4% Agar gel.

**Colour and Clarity of prepared medium**

Amber coloured, clear to slightly opalescent gel forms in Petri plates

**Reaction**

Reaction of 5.8% w/v aqueous solution at 25°C. pH : 6.9±0.2

**Cultural Response**

M433: Cultural characteristics observed with added 4-5% defibrinated sheep blood after an incubation at 35-37°C for 48-72 hours in presence of 10% CO<sub>2</sub>

Organism	Growth					
<i>Francisella tularensis</i> ATCC 29684	luxuriant					

**Reference**

- 1.Murray P. R., Baron J. H., Pfaller M. A., Tenover J. C. and Tenover F. C., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
- 2.Collee J. G., Marmion B. P., Fraser A. G., and Simmons A., (Eds.), Mackie and McCartney Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone, New York.
- 3.Manual of Diagnostic Tests and Vaccine for Terrestrial Animals, 2004, 5th Edi, OIE World Organization for Animal Health.
- 4.Atlas R. M., 2004, Handbook of Microbiological Media, Lawrence C. Parks, (Ed.), 3rd Edition, CRC Press, pg. no 717.

**Storage and Shelf Life**

Store below 30°C and prepared medium at 2-8°C. Use before expiry period on the label.