Cystine Tryptone Agar M159

Cystine Tryptone Agar is recommended for maintenance, subculturing, detection of motility etc. With added carbohydrates, it can be also used for fermentation reactions of fastidious organisms.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	20.000
L-Cystine	0.500
Sodium chloride	5.000
Sodium sulphite	0.500
Phenol red	0.017
Agar	2.500
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 28.51 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes in 8-10 ml amounts. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and add appropriate carbohydrate (0.5 to 1.0% if desired). Mix well and allow the tubed medium to cool in an upright position.

Principle And Interpretation

Cystine Tryptone Agar appropriates for the propagation and maintenance of bacteria even the fastidious ones without addition of additives. This formulation was developed by Vera as a simple semisolid medium for the identification and maintenance of the *Gonococcus* and other bacteria (1). In this medium by deep stab, a lot of cultures can be maintained including fastidious organisms like *Brucella, Corynebacterium, Pasteurella, Pneumococci* and *Streptococci* without added enrichments (2, 3, 4) for longer periods when stored at appropriate temperatures. Even some light-sensitive anaerobic microorganisms can grow in this medium without special conditions though in reduced atmospheres, they give ideal growth. This medium has its maximum efficiency when freshly prepared, but it can be stored for long period of time, taking care to avoid dehydration. To achieve this, screw caps or proper sealing are strongly recommended. Anaerobic organisms like *Actinomyces bovis, Bacteroides funduliformis* and *Leptotrichia* (5) grow well in this medium in presence of CO_2. With added carbohydrate, it may be used to study sugar fermentation of microorganisms that do not grow on phenol red classical media. Acidification can be easily observed with the change in colour of phenol red indicator. In semisolid agar, acid reactions are easily detected because the acid formed is not immediately diffused throughout the entire culture as in broth. Most cultures show an alkaline reaction when no fermentable carbohydrate is present. Motility can be readily detected in the semisolid medium (6). Motile cultures show growth away from the line of inoculation. Non-motile organisms grow in the inoculated area, along the stab line while the surrounding area remains clear.

Casein enzymic hydrolysate, L-cystine supplies the nutrients necessary to support the growth of fastidious microorganism. Carbohydrate fermentation is detected by a visible colour change of the medium due to the incorporation of the pH indicator dye, phenol red. When the organism metabolizes the carbohydrate present, organic acids are produced and the medium becomes acidified. However, the peptones present in the medium are also degraded by the bacteria present and yield substances that are alkaline in pH. The phenol red indicator changes from reddish-orange to yellow when the amount of acid produced by carbohydrate fermentation is greater than the alkaline end products of the peptone degradation. The colour change with phenol red occurs around pH 6.8, near the original pH of the medium.

Only the surface of the tubed medium is inoculated in case of fermentation studies of the genus *Neisseria* . For facultative organisms, such as Streptococci and strictly anaerobic organisms inoculation is done by stabbing the center of the medium with an inoculating needle to about ½ the depth of the medium. Incubate with loosened caps aerobically or anaerobically depending upon the organisms being tested. *Neisseria* should be incubated with loose caps (10); if incubated in CO_2 incubator (11, 12) or with tight caps in non-CO_2 incubator (13). For more rapid growth and also for more rapid fermentation reactions, anaerobic cultures preferably should be incubated in the presence of CO_2 as well as hydrogen or nitrogen. Some strict anaerobes fail to grow or grow poorly in the absence of CO_2.

A yellow colour either in the upper one-third or throughout the medium indicates acid production due to carbohydrate fermentation. A red (alkaline) to orange (neutral) colour indicates that the carbohydrate has not been degraded and that only the peptone has been utilized. Inoculated medium (without carbohydrate) also exhibits a red to orange colour.

This medium requires a heavy inoculum (7). Prolonged incubation may lead to changes in pH indicator or abnormal lactose/sucrose reactions with *Neisseria* pathogens (8, 9). *Neisseria* species usually produce acid only in the area of stabs (upper third). If there is a strong acid (yellow color) throughout the medium, a contaminating organism may be present. Gram stain and oxidase test should be performed on the growth to confirm the presence of *Niesseria* species (7).

Quality Control

Appearance

Light yellow to light pink homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.25% Agar gel.

Colour and Clarity of prepared medium

Red coloured, clear to slightly opalescent gel forms in tubes as butts

Reaction

Reaction of 2.85% w/v aqueous solution at 25°C. pH : 7.3±0.2

Cultural Response

M159: Cultural characteristics observed after an incubation at 35-37°C for 4-18 hours or longer if necessary.

Organism	**Inoculum (CFU)**	**Growth**	**Motility**	**Acid in presence of Dextrose**		
Escherichia coli ATCC 25922	50-100	good-luxuriant	positive, growth away from stabline causing turbidity	positive reaction, yellow colour		
Neisseria gonorrhoeae ATCC 19424	50-100	good	negative, growth along the stabline, surrounding medium remains clear	positive reaction, yellow colour		
Neisseria meningitidis ATCC 13090	50-100	good	negative, growth along the stabline, surrounding medium remains clear	positive reaction, yellow colour		
Streptococcus pneumoniae ATCC 6303	50-100	good	negative, growth along the stabline, surrounding medium remains clear	positive reaction, yellow colour		

Reference

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Storage and Shelf Life

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