



## Tryptone Sucrose Tetrazolium Agar Base (TSTA)

M1217

Tryptone Sucrose Tetrazolium Agar Base (TSTA) with addition of Triphenyl Tetrazolium Chloride is recommended for isolation of *Vibrio* species.

### Composition\*\*

Ingredients	Gms / Litre
Casein enzymic hydrolysate	15.000
Papaic digest of soyabean meal	5.000
Sodium chloride	30.000
Saccharose	20.000
Bile salts	0.500
Agar	15.000
Final pH ( at 25°C)	7.1±0.2

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

### Directions

Suspend 85.5 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 3 ml of 1% 2, 3, 5-Triphenyl Tetrazolium Chloride (TTC) (FD057). Mix well before pouring into sterile Petri plates.

### Principle And Interpretation

The *Vibrionaceae* are straight or curved, gram-negative rods, motile by polar flagella. Many strains require 2-3% sodium chloride for growth and are primarily inhabitants of aquatic environments. Of the 35 *Vibrio* species recognized, 12 have been implicated in gastrointestinal and extra-intestinal infections in man; the most important of these is cholera. The species most frequently isolated from clinical specimens are strains of *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio mimicus* and *Vibrio alginolyticus* (1).

Tryptone Sucrose Tetrazolium Agar is formulated in accordance with Kourany medium (2) and is approved by ISO Committee (3) for the isolation of *Vibrio* species, especially *V. parahaemolyticus*. Casein enzymic hydrolysate and papaic digest of soyabean meal provide nitrogenous compounds and other essential growth nutrients. Saccharose (sucrose) is the energy source. High salt concentration makes it specific for organisms having high osmotic tolerance. Bile salts inhibit gram-positive organisms. TTC is reduced by *V. parahaemolyticus* to red formazan dyes, visualized as red colonies.

Inoculate 25 grams of the test sample into 225ml of Salt Polymyxin Broth Base (M821I). Incubate at 35-37°C for 7 to 8 hours. After incubation, inoculate a loopful onto TCBS Agar (M189) & Tryptone Sucrose Tetrazolium Agar Base (M1217). Presumptive *V. parahaemolyticus* colonies are further confirmed by appropriate biochemical tests.

### Quality Control

#### Appearance

Cream to yellow homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.5% Agar gel

#### Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in petriplates.

#### Reaction

Reaction of 8.55% w/v aqueous solution at 25°C. pH : 7.1±0.2

#### pH

6.90-7.30

#### Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

## Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery
<b>Cultural Response</b> <i>Vibrio parahaemolyticus</i> ATCC 17802	50-100	good-luxuriant	>=50%
<i>Vibrio cholerae</i> ATCC 15748	50-100	good-luxuriant	>=50%

## 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. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone
2. Kourany M., 1983, Appl. Environ. Microbiol., 45: 310.3. International Organization for Standardization (ISO) 1990, Draft, ISO/DIS 8914.

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