



TCBS AGAR

- For in vitro use only -

Catalogue No. PT25

Our Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS Agar) is used for the selective isolation of cholera vibrios and *Vibrio parahaemolyticus* from a variety of clinical and nonclinical specimens.

Vibrio species are most widely recognized for their role in human intestinal infections, and cholera and *V. parahaemolyticus* diarrhea are important worldwide. The isolation and cultivation of *Vibrio* species has been enhanced by the development of media which are highly selective for vibrios. TCBS Agar is the primary plating medium universally used for the selective isolation of vibrios causing cholera, diarrhea and food poisoning. It was developed by Kobayashi et al., who modified the selective medium of Nakanishi.

Our TCBS Agar is highly selective for the isolation of *V. cholerae* and *V. parahaemolyticus* as well as other vibrios. Inhibition of gram-positive bacteria is achieved by the incorporation of the bile salts, oxgall and sodium cholate. Sodium thiosulfate serves as a sulfur source and, in combination with ferric ammonium citrate, detects hydrogen sulfide production. Sucrose is included as a fermentable carbohydrate for the metabolism of vibrios. The alkaline pH of the medium enhances the recovery of *V. cholerae*. Thymol blue and bromthymol blue are included as indicators of pH changes.

Formula per Litre of Medium

Proteose Peptone No.3.....	10.0 g
Yeast Extract	5.0 g
Sodium Citrate	10.0 g
Sodium Thiosulfate.....	10.0 g
Oxgall	8.0 g
Saccharose.....	20.0 g

Sodium Chloride.....	10.0 g
Ferric Ammonium Citrate	1.0 g
Bromothymol Blue	0.04 g
Thymol Blue	0.04 g
Agar.....	15.0 g

pH 8.6 ± 0.2

Recommended Procedure

1. Allow medium to reach room temperature.
2. Using an inoculum from the specimen, perform a four-quadrant streak to obtain well-isolated colonies. Alternatively, if the specimen is contained on a swab, streak the specimen as soon as possible after it is received in the laboratory. Specimens such as rectal swabs, feces, vomitus, fish or food samples may be swabbed directly onto the plated medium. Heavy inoculation is recommended, especially if specimens are not fresh, as the medium is highly selective and vibrios tend to die rather easily.
3. Incubate at 35°C in an aerobic environment.
4. Examine after 18-24 hours. Incubate an additional 24 hours if required.

Interpretation of Results

After incubation, most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a dilution technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Better isolation is obtained due to the inhibitory action of the medium.

Typical colonial morphology on TCBS Agar is as follows:

V. choleraeLarge yellow colonies
V. parahaemolyticusColonies with blue-green centers
V. alginolyticus.....Large yellow colonies
Proteus/EnterococciPartial inhibition
 If growth, colonies are small and yellow to translucent
Pseudomonas/Aeromonas.....Partial inhibition
 If growth, colonies are blue

Additional biochemical and/or serological tests should be performed on isolated colonies from pure culture in order to complete identification.

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Results
<i>Vibrio parahaemolyticus</i> ATCC 17802	Growth, blue green
<i>Enterococcus faecalis</i> ATCC 29212	Inhibition
<i>Escherichia coli</i> ATCC 25922	Inhibition

Storage and Shelf Life

Our TCBS Agar should be stored away from direct light at 4 to 8°C. The medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of 8 weeks from the date of manufacture.

References

1. Kobayashi T, Enomoto S, Sakazaki R, Kuwahara S. A new selective medium for pathogenic vibrios, TCBS (modified Nakanishi's agar). Jpn J Bacteriol 1963; 18:387.
2. Nakanishi Y. An isolation agar medium for cholerae and enteropathogenic halophilic vibrios. Modern Media 1963; 9:246.
3. MacFaddin JF. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol I. Baltimore, MD: Williams & Wilkins, 1985.
4. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostic microbiology. 10th ed. St Louis: Mosby, 1998.
5. Downes, Ito. Compendium of methods for the microbiological examination of foods. 4th ed. Washington, DC: APHA, 2001.
6. Difco & BBL Manual. 2nd ed. Sparks, Maryland: BD and Company, 2009.

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