

TCBS Agar

Intended Use

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.^{1,2}

Summary and Explanation

Vibrio species are most widely recognized for their role in human intestinal infections. Diarrheas caused by *Vibrio cholerae* and *V. parahaemolyticus* are important worldwide.³ The isolation of *Vibrio* species has been enhanced by the development of media which are highly selective for vibrios.

TCBS is the primary plating medium universally used for the selective isolation of vibrios that cause cholera, diarrhea and food poisoning. It was developed by Kobayashi et al.⁴, who modified the selective medium of Nakanishi.⁵ The combination of alkaline peptone water and TCBS Agar is used in many procedures for the isolation of *V. cholerae* and other *Vibrio* species from feces.^{1-3,6,7}

TCBS Agar Deep (pour tubes) are provided in a 20 mL fill so that the medium may be liquefied and poured into a Petri dish. This provides a convenient source of medium with a longer shelf-life than pre-poured plated media.

Principles of the Procedure

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 oxgall, which is a naturally occurring substance containing a mixture of bile salts, and sodium cholate, a pure bile salt. Sodium thiosulfate serves as a sulfur source and, in combination with ferric citrate, detects hydrogen sulfide production. Saccharose (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

Difco™ TCBS Agar

Approximate Formula* Per Liter	
Yeast Extract	5.0 g
Proteose Peptone No. 3.....	10.0 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
Bromthymol Blue	0.04 g
Thymol Blue	0.04 g
Agar	15.0 g

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

1. Suspend 89 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Cool to 45-50°C and use immediately. DO NOT AUTO-CLAVE.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

To prepare plated media, place agar deeps with caps loosened in a boiling water bath until the medium becomes liquefied. Pour the molten medium into a sterile Petri dish. Allow the medium to solidify. Store the plates, protected from light, in an inverted position (agar side up) at 2-8°C until ready to use.

User Quality Control

Identity Specifications

Difco™ TCBS Agar

Dehydrated Appearance:	Light tan with greenish cast, free-flowing, homogeneous.
Solution:	8.9% solution, soluble in purified water upon boiling. Solution is forest green, very slightly opalescent.
Prepared Appearance:	Green, slightly opalescent.
Reaction of 8.9% Solution at 25°C:	pH 8.6 ± 0.2

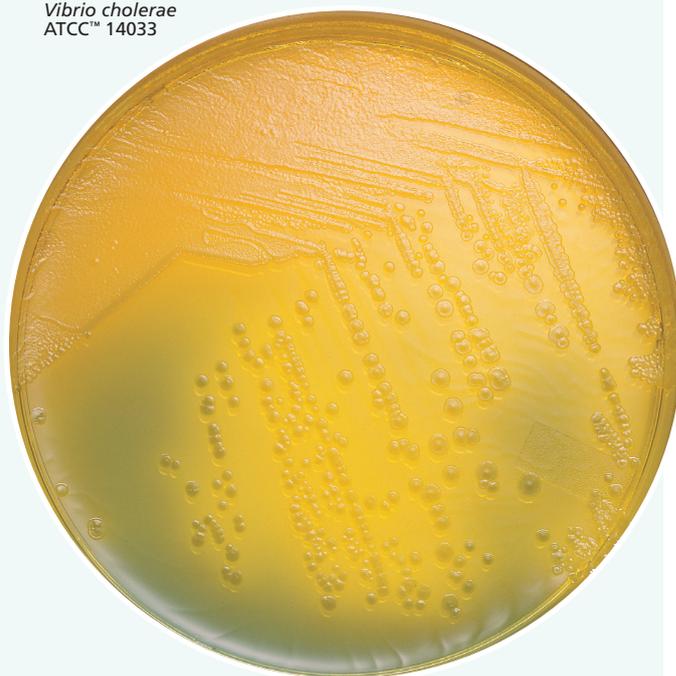
Cultural Response

Difco™ TCBS Agar

Prepare the medium per label directions. Inoculate with fresh cultures (*E. coli* grown in TSB; vibrios grown in BHI) and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	RECOVERY	COLONY COLOR
<i>Escherichia coli</i>	25922	None	–
<i>Vibrio alginolyticus</i>	17749	Good	Yellow
<i>Vibrio cholerae</i> El Tor	14033	Good	Yellow
<i>Vibrio parahemolyticus</i>	17802	Good	Blue green

Vibrio cholerae
ATCC™ 14033



Use standard procedures to obtain isolated colonies from specimens. Incubate the plates, protected from light, in an inverted position (agar side up) at 35°C for 24-48 hours.

Expected Results

Typical colonial morphology on TCBS Agar is as follows:

<i>V. cholerae</i>	Large yellow colonies.
<i>V. parahaemolyticus</i>	Colonies with blue to green centers.
<i>V. alginolyticus</i>	Large yellow colonies.
<i>Proteus/Enterococci</i>	Partial inhibition. If growth, colonies are small and yellow to translucent.
<i>Pseudomonas/Aeromonas</i>	Partial inhibition. If growth, colonies are blue.

Limitations of the Procedure

1. On initial isolation, *V. parahaemolyticus* may be confused with *Aeromonas hydrophila*, *Plesiomonas shigelloides* and *Pseudomonas* species.⁸
2. Sucrose-fermenting *Proteus* species produce yellow colonies which may resemble those of *Vibrio*.⁹
3. TCBS is an unsatisfactory medium for oxidase testing of *Vibrio* spp.¹⁰
4. A few strains of *V. cholerae* may appear green or colorless on TCBS due to delayed sucrose fermentation.⁹

References

1. Downes and Ito. 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
2. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed, online. American Public Health Association, Washington, D.C.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
4. Kobayashi, Enomoto, Sakazaki and Kuwahara. 1963. Jap. J. Bacteriol. 18: 387.
5. Nakanishi. 1963. Modern Media 9: 246.
6. Furniss, Lee and Donovan. 1978. The vibrios. Public Health Laboratory Service Monograph Series no. 11. Maidstone Public Health Laboratory. H.M.S.O., London, England.
7. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc. St. Louis, Mo.
8. Bottone and Robin. 1978. J. Clin. Microbiol. 8:760.
9. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
10. Morris, Merson, Huq, Kibrya and Black. 1979. J. Clin. Microbiol. 9:79.

Availability

Difco™ TCBS Agar

AOAC BAM BS12 CCAM CMPH2 COMPF ISO MCM9 SMWW

Cat. No. 265020 Dehydrated – 500 g

BBL™ TCBS Agar

AOAC BAM BS12 CCAM CMPH2 COMPF ISO MCM9 SMWW

United States and Canada

Cat. No. 221872 Prepared Plates – Pkg. of 10*
297437 Prepared Pour Tubes, 20 mL – Pkg. of 10*

Europe

Cat. No. 254432 Prepared Plates – Pkg. of 20*

Japan

Cat. No. 251143 Prepared Plates – Pkg. of 20*
251137 Prepared Plates – Ctn. of 100*
251509 Prepared **RODAC™** Plates – Pkg. of 30*

Mexico

Cat. No. 226850 Prepared Plates – Pkg. of 10*

*Store at 2-8°C.