TCBS AGAR (7210)

Intended Use
TCBS Agar is used for the selective isolation of *Vibrio cholerae* and other enteropathogenic vibrios.

Product Summary and Explanation
TCBS Agar is an abbreviation for Thiosulfate-Citrate-Bile-Sucrose Agar. TCBS Agar is also called Vibrio Selective Agar, and prepared according to the formula of Kobayashi et al.[1] This formula is a modification of the selective medium from Nakanishi.[2] All pathogenic *Vibrio* spp., except *Vibrio hollisae*, will grow on TCBS Agar. This highly selective agar meets the nutritional requirements of *Vibrio* spp., and allows vibrios to compete with intestinal flora. *Vibrio* spp. are able to grow in media containing increased salt concentrations, and some species are halophilic.[3] Infections have been associated with ingestion of contaminated water and consumption of contaminated shellfish or seafood. *Vibrio* spp. are natural inhabitants of seawater.[3]

TCBS Agar is recommended for isolating *Vibrio* spp. from stool specimens,[4] and specified in standard methods for food testing.[5,6]

Principles of the Procedure
Yeast Extract, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue provides the nitrogen, vitamins, and amino acids in TCBS Agar. Sodium Citrate, Sodium Thiosulfate, Sodium Cholate, and Oxbile are selective agents, providing an alkaline pH to inhibit Gram-positive organisms and suppress coliforms. An increased pH is used to enhance growth of *Vibrio cholerae,* because this organism is sensitive to acid environments. Sucrose is the fermentable carbohydrate. Sodium Chloride stimulates organism growth and maintains osmotic balance of the medium. Sodium Thiosulfate is also a sulfur source, and acts with Ferric Citrate as an indicator to detect hydrogen sulfide production. Bromthymol Blue and Thymol Blue are pH indicators. Agar is the solidifying agent.

Formula / Liter
Yeast Extract	5 g
Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue	5 g
Sodium Citrate	10 g
Sodium Thiosulfate	10 g
Oxbile	5 g
Sodium Cholate	3 g
Sucrose	20 g
Sodium Chloride	10 g
Ferric Citrate	1 g
Bromthymol Blue	0.04 g
Thymol Blue	0.04 g
Agar	14 g

Final pH: 8.6 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions
1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 88 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing, and beige to green-beige.

Prepared Appearance: Prepared medium is forest green and clear to trace hazy.

Expected Cultural Response: Cultural response on TCBS Agar at 35°C after 18 - 24 hours of incubation.

Microorganism	Response	Reactions
Enterococcus faecalis ATCC® 29212	inhibited	---
Escherichia coli ATCC® 25922	inhibited	---
Vibrio alginolyticus ATCC® 17749	growth	yellow colonies
Vibrio cholerae ATCC® 9459	growth	yellow colonies
Vibrio parahaemolyticus ATCC® 17802	growth	green colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion on the isolation and identification of *Vibrio cholerae* and other enteropathogenic vibrios, refer to specific procedures.

Results

After 18 – 24 hours of incubation at 35°C, sucrose-fermentating vibrios (*V. cholerae, V. alginolyticus, V. hareyi, V. cincinnatiensis, V. fluvialis, V. furnissii, V. metschnikovii,* and some *V. vulnificus*) appear smooth, opaque, thin-edged yellow colonies on TCBS Agar.[3]

Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Dehydrated medium should be discarded if not free flowing, or if appearance has changed from original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. Further tests are necessary for confirmation of *Vibrio* spp.
2. On initial isolation, *V. parahaemolyticus* may be confused with *Aeromonas hydrophila*, *Plesiomonas shigelloides,* and *Pseudomonas* spp.[7]
3. Sucrose-fermenting *Proteus* spp. produce yellow colonies which may resemble those of *Vibrio*.[8]
4. TCBS is an unsatisfactory medium for oxidase testing of *Vibrio* spp.[9]
5. A few strains of *V. cholerae* may appear green or colorless on TCBS Agar due to delayed sucrose.fermentation.[8]

Packaging

TCBS Agar	**Code No.**	**7210A**	**500 g**
		7210B	**2 kg**
		7210C	**10 kg**

References

1. **Kobayashi, T., S. Enomoto, R. Sakazaki, and S. Kuwahara.** 1963. A new selective medium for pathogenic vibrios, TCBS (modified Nakanishi's agar). Jpn, J. Bacteriol. **18**:387.
2. **Nakanishi, Y.** 1963. An isolation agar medium for cholerae and enteropathogenic halophilic vibrios. Modern Media. **9**:246.
3. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. *Vibrio* and related species, *Aeromonas, Plesiomonas, Campylobacter*, *Helicobacter*, and others, p. 429-444. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
6. **Vanderzant, C. and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food. 3rd ed. American Public Health Association, Washington, D.C.
7. **Bottone, E. J., and T. Robin.** 1978. *Vibrio parahaemolyticus*; Suspicion of presence based on aberrant biochemical and morphological features. J. Clin. Microbiol. **8**:760.
8. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol.1, p. 763-767. Williams & Wilkins, Baltimore, MD.
9. **Morris, G. K., M. H. Merson, I. Huq, A. Kibrya, and R. Black.** 1979. Comparison of four plating media for isolating *Vibrio cholerae*. J. Clin. Microbiol. **9**:79.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.