

Thiosulfate Citrate Bile Salts Sucrose (TCBS) Agar (NCM0052)

Intended Use

Thiosulfate Citrate Bile Salts Sucrose (TCBS) Agar is used for the selective isolation of *Vibrio cholerae* and other enteropathogenic vibrios according to ISO 21872-1:2017 and ISO 21872-2:2007. Thiosulfate Citrate Bile Salts Sucrose (TCBS) Agar is not intended for use in the diagnosis of disease or other conditions in humans.

Description

TCBS Agar is also called Vibrio Selective Agar, and prepared according to the formula of Kobayashi et al. This formula is a modification of the selective medium from Nakanishi. All pathogenic *Vibrio* spp., except *Vibrio hollisae*, will grow on TCBS Agar. This highly selective agar meets the nutritional requirements of *Vibrio* spp. *Vibrio* spp. are able to grow in media containing increased salt concentrations, and some species are halophilic. Infections have been associated with ingestion of contaminated water and consumption of contaminated shellfish or seafood. *Vibrio* spp. are natural inhabitants of seawater.

According to ISO 21872-2, pre-incubation in Alkaline Saline Peptone Water (ASPW) (NCM0175) is required.

Typical Formulation

Yeast Extract	5.0 g/L
Enzymatic Digest of Casein	5.0 g/L
Enzymatic Digest of Animal Tissue	5.0 g/L
Sodium Citrate	10.0 g/L
Sodium Thiosulfate	10.0 g/L
Bile Salts	8.0 g/L
Sucrose	20.0 g/L
Sodium Chloride	10.0 g/L
Ferric Citrate	1.0 g/L
Bromothymol Blue	0.04 g/L
Thymol Blue	0.04 g/L
Agar	*14.0 g/L

*10 - 15 g according to gel strength

Final pH: 8.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

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 medium.
3. DO NOT AUTOCLAVE

Test Procedure

1. For a complete discussion on the isolation and identification of *Vibrio cholerae* and other enteropathogenic *Vibrio* spp., refer to specific procedures, such as ISO 21872-1:2017 and ISO 21872-2:2007.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and greenish beige to light beige.



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

Expected Cultural Response: Cultural response on TCBS Agar at 36-38°C after 21-27 hours of incubation.

Microorganism	Approx. Inoculum (CFU)	Response	Reaction
<i>Enterococcus faecalis</i> ATCC® 29212	>10 ⁴	Markedly Suppressed to Inhibited	If recovered, small translucent colonies
<i>Escherichia coli</i> ATCC® 25922	>10 ⁴	Complete Inhibition	---
<i>Escherichia coli</i> ATCC® 8739	>10 ⁴	Complete Inhibition	---
<i>Escherichia coli</i> ATCC® 11775	>10 ⁴	Complete Inhibition	---
<i>Salmonella typhimurium</i> ATCC® 14028	>10 ⁴	Complete Inhibition	---
<i>Vibrio alginolyticus</i> ATCC® 17749	~1000	Growth	Yellow
<i>Vibrio cholera</i> ATCC® 14733	~1000	Growth	Yellow
<i>Vibrio furnissii</i> NCTC 11218	~1000	Growth	Yellow
<i>Vibrio parahaemolyticus</i> ATCC® 10885	~1000	Growth	Green

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

Results

After 18 – 48 hours of incubation at 35 ± 2°C, sucrose-fermentating *Vibrio* spp. (*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.

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.

Storage

Store dehydrated culture media at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. ISO 21872-1:2017 Microbiology of the food chain – Horizontal method for the determination of *Vibrio* spp. – Part 1: Detection of potentially enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*.
2. ISO 21872-2:2007 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. – Part 2: Detection of species other than *Vibrio parahaemolyticus* and *Vibrio cholerae*.
3. 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.
4. Nakanishi, Y. 1963. An isolation agar medium for cholerae and enteropathogenic halophilic vibrios. *Modern Media.* 9:246.
5. 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.
6. 4. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm.



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7. Vanderzant, C. and D. F. Splittstoesser (eds.). 2015. Compendium of methods for the microbiological examination of food. 4th ed. American Public Health Association, Washington, D.C. 6.

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