

Also known as

Cholera Medium TCBS

Specification

 Solid medium for the selective isolation of *Vibrio spp.*, and *Vibrio parahaemolyticus* according to the ISO standard.

Formula * in g/L

Proteose peptone.....	10.000		
Yeast extract.....	5.000	Ferric citrate.....	1.000
Sodium citrate.....	10.000	Thymol blue.....	0.040
Sodium thiosulphate.....	10.000	Bromthymol blue.....	0.040
Ox bile.....	8.000	Agar.....	14.000
Sucrose.....	20.000		
Sodium chloride.....	10.000		

Final pH 8,6 ±0,2 at 25 °C

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

Directions

Suspend 88 g of powder in 1 l of purified water. Heat with constant stirring until boiling. Pour immediately into plates. Do not sterilise and avoid remelting.

Description

 TCBS Agar is universally accepted as the medium of choice for differential isolation of enteropathogenic vibrios, whilst inhibiting all the accompanying organisms. This formulation provides high growth of *Vibrio cholerae* and *V. parahaemolyticus*. *V. alginolyticus* and NAG-vibrios. Enterobacteria are strongly inhibited by high concentrations of citrate, thiosulfate, bile and sodium chloride.

 Although some enteric bacteria may also grow in this medium, their colony morphology is quite different to that of *Vibrio spp.*

 The organisms that can be confused with vibrios are some biotypes of *Proteus* and *Pseudomonas*. There are some resistant enterococci which may form exceptionally small and yellow colonies on this medium. Usually, colonies are selected or chosen and then identified with primary tests [oxidase reactions in Kligler Iron Agar, MRVP Broth, and antibiotic sensitivity test] before performing serological identification and phage typing.

Due to its high selectivity, the medium can be seeded with large inoculum of pathological material. Once solidified and cooled, the medium is turbid, but the observations are not affected.

This medium is very thermolabile and so it must not be autoclaved, overheated or re-melted.

Colonial appearance on TCBS Agar after 24 hours at 37°C:

- *Vibrio alginolyticus* and *Vibrio cholerae*: Large, yellow colonies.
- *Vibrio parahaemolyticus*: Small, yellow, without halo and with a green core.
- *Streptococcus faecalis*: Very small and convex, yellow with yellow halo.
- Enterobacteria generally: Small and transparent.
- *Pseudomonas*, *Aeromonas*, *Proteus*: sized and blue colonies.
- Some strains of *Vibrio cholerae* and *Vibrio parahaemolyticus* carry out delayed sucrose fermentation so they produce medium sized colonies, and are colourless or dirty yellow with a dark nucleus.

Quality control
Incubation temperature: 37°C ±1.0

Incubation time: 24±3 h

Inoculum: Previous enrichment. 6±1h (ASPW). Streak isolation. (ISO 11133:2014/Amd 1:2018)

Microorganism	Growth	Remarks
<i>Vibrio parahaemolyticus</i> ATCC® 17802	Good	Blue-green colonies 3-5mmØ
<i>Vibrio alginolyticus</i> ATCC® 17749	Good	Yellow colonies 3-5mmØ
<i>Vibrio furnissii</i> NCTC® 11218	Good	Yellow colonies 3-5mmØ
<i>Escherichia coli</i> ATCC® 8739	Inhibited	-

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media CRC Press. BocaRaton. Fla. USA.
- BHATTACHARYA, M.K., S.K. BATTACHARYA, S. GARG, P.K. SAHA, D. DUTTA, G.B. NAIR, B.C. DEB & K.P. DAS (1993) Outbreak of *Vibrio cholerae* non-01 in India and Bangladesh. *Lancet*, 341:1345-1347.
- DOWNES, F.P. & K. ITO (2001). Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington. DC. USA.
- FORBES, B.A., D.F SAHM & A.S. WEISSFELD (Eds) (1998) Bailey & Scott's Diagnostic Microbiology 10th ed. Mosby. St. Louis, MO. USA.
- HORWITZ, W. (Ed) (2000) Official Methods of Analysis of AOAC International. 17th ed. Gaithersburg. MD. USA.
- ISO 21872-1 Technical Specification (2017) Microbiology of Food chain- Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. - Part 1: Detection of *Vibrio parahaemolyticus* and *Vibrio cholerae* and *Vibrio vulnificus*.
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- KOBAYASHI, T., ENOMOTO, S. SAKAZARI, R. and KUWAHARA, S. (1963) A new selective medium for pathogenic vibrios: TCBS (modified Nakanishi Agar) *Jap. J.Bact.* 18:387.
- MacFADDIN, J.F. (1985) Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins. Baltimore. MD. USA.
- MURRAY, P.R., E.J. BARON, J.H. JORGENSEN, M.A. PFALLER & R.H. YOLKEN (Eds) (2003) Manual of Clinical Microbiology 8th ed. ASM Press. Washington. DC. USA.
- PASCUAL ANDERSON, M^ªR^a (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.
- US FDA (Food and Drug Administration) (1998) Bacteriological Analytical Manual 8th ed. Revision A. AOAC International Inc. Gaithersburg. MD. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).