

TCBS Agar ISO

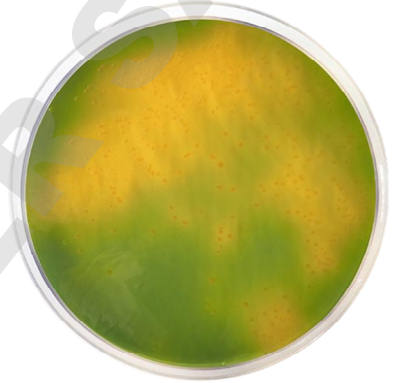
For the selective isolation of *Vibrio* from a variety of clinical samples and other materials

Practical information

Applications	Categories
Selective isolation	Vibrio

Industry: Clinical / Food

Regulations: ISO 21872



Principles and uses

TCBS Agar is a selective medium widely used to isolate and cultivate practically all bacteria of the genus *Vibrio*, including *V. cholerae* and *V. alginolyticus*, pathogenic to humans causing cholera, choleral diarrhea or food poisoning from contaminated foods and from stool specimens. The last 2 conditions especially can be caused by ingesting raw or partially processed fish or seafood containing *Vibrio parahaemolyticus*. The only *Vibrio* that does not grow in TCBS is *V. hollisae*.

The Meat and Casein peptones provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Sodium citrate, Sodium thiosulfate and Ox bile are the selective agents, inhibiting the Gram positive bacteria. Sodium thiosulfate provides sulphur, and Ferric citrate is the indicator for H₂S production. Sucrose is the carbohydrate energy source. Bromothymol blue and Thymol blue are pH indicators. Sodium chloride promotes growth (*Vibrio* grows well in salty media). Bacteriological agar is the solidifying agent. The alkaline pH of the medium enhances the recovery of *V. cholerae*.

Sucrose-positive vibrios, such as *Vibrio cholerae* and *Vibrio alginolyticus*, are yellow on TCBS. Sucrose-negative ones, such as *Vibrio parahaemolyticus* and *Vibrio vulnificus*, produce blue- green colonies. Almost all *Vibrio* ferment sucrose and yield yellow colonies from the production of acid. Some types of *Proteus* (fermenters of sucrose) can form yellow colonies similar to those of *Vibrio*.

Formula in g/L

Bromthymol blue	0,04	Bacteriological agar	14
Peptone	10	Sodium chloride	10
Sodium citrate	10	Sodium thiosulfate	10
Sucrose	20	Thymol blue	0,04
Yeast extract	5	Ferric citrate	1
Bovine bile	8		

Preparation

Suspend 88 grams of the medium in one liter of distilled water. Mix well. Dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. DO NOT OVERHEAT. DO NOT AUTOCLAVE. Cool to 50 °C, mix well and dispense into plates.

Instructions for use

- » For clinical diagnosis, the type of sample is feces, vomit and rectal swabs.
- Inoculate on the surface. Parallel striae with the handle or swab.
- Incubate in aerobic conditions at 35±2 °C for 18-24 hours.
- Reading and interpretation of the results.

» For other uses not covered by the CE marking:

Detection of potentially enteropathic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus* according to ISO 21872:

- Take test portions (25 g or 25 ml) and homogenize in 225 ml of enrichment medium ASPW. In the case of large quantities of test portion, the ASPW should be warmed to 37±1 °C / 41,5±1 °C before inoculation.
- Incubate the initial suspension at 41,5±1 °C / 37±1 °C for 6±1 hours.
- Transfer 1 ml from the surface into a tube with 10 ml of ASPW.
- Incubate the ASPW at 41,5±1 °C / 37±1 °C for 18±1 hours.
- From the culture obtained in the ASPW, inoculate 1 µl in TCBS Agar. Incubate a second selective isolation medium.
- Incubate at 37±1 °C for 24±3 hours.
- Confirmation.

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Light toasted with a green tint	Green	8,6±0,2

Microbiological test

According to ISO 11133:

Incubation conditions: (37±1 °C / 24±3 h).

Inoculation conditions: Productivity qualitative (10³-10⁴ CFU) / Selectivity (10⁴-10⁶ CFU).

Microrganisms	Specification	Characteristic reaction
<i>Vibrio parahaemolyticus</i> NCTC 10885	Good growth (2)	Green colonies (sucrose negative)
<i>Vibrio furnissi</i> NCTC 11218	Good growth (2)	Yellow colonies (sucrose positive)
<i>Escherichia coli</i> ATCC 25922	Total inhibition (0)	

Storage

Temp. Min.: 2 °C

Temp. Max.: 25 °C

Bibliography

Cholera Information (WHO, 1965). WHO Expert Committee on Cholera (2 and Rep. Techn., Rep. Series No. 352. 1967. Felsemfeld, Bull.

World Otg. 34:161. 1966. Kobayashi. T. Enomoto S. Sakasaki, R. Y.

Kwajaras, S., Jap. J. Bact. 18 387 291. 1963.

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*