

TCBS Agar

For the selective isolation of vibrium

Formula in grams per liter:

Yeast Extract	5,00	Casein Peptone	5,00
Meat Peptone	5,00	Sodium Citrate	10,00
Sodium Thiosulfate	10,00	Oxbile	5,00
Sodium Cholate	3,00	Sucrose	20,00
Sodium Chloride	10,00	Ferric Citrate	1,00
Thymol Blue	0,04	Bromothymol Blue	0,04
Bacteriological Agar	14,00		

Final pH: 8,6 ± 0,2 at 25 °C

Preparation:

Suspend 88 grams of the medium in one litre of distilled water. Mix from 10 to 15 minute. Heat with frequent agitation and boil for 1 minute until completely dissolved. Cool to 45-50°C and pour in Petri dishes. **Do not sterilize in an autoclave.**

Uses:

TCBS Agar is widely used to isolate and cultivate diverse species of the genus *Vibrio* that can cause cholera, choleral diarrhea or food poisoning from contaminated foods. The last 2 conditions especially can be caused by ingesting raw or partially processed fish or seafood containing *Vibrio parahaemolyticus*.

TCBS Agar is highly inhibitory to the enterobacteria, including coliforms and *Proteus*. Enterococci are also greatly inhibited and allows the proliferation of vibrio, such as *V. cholerae* and *V. alginolyticus*. The suspect material (feces, vomit, rectal swabs, fish, and other food), is heavily inoculated on the surface of the plate, incubated at 35°C for 18 to 24 hours.

Almost all vibrios ferment sucrose and yield yellowish colonies from the production of acid. Some types of *Proteus* (fermenters of sucrose) can form yellowish colonies similar to those of vibrios.

MICROORGANISMS	CHARACTERISTICS OF THE COLONIES
<i>Vibrio cholera</i> and its biotype <i>Tor</i>	Large, smooth, elevated, yellow or pale yellowish brown. 2 - 3 mm. in diameter. Yellow agar.
<i>V. parahaemolyticus</i> (GROUP I)	Colorless with a green center. 3 - 4 mm in diameter. No color in agar.
<i>V. Parahaemolyticus</i> (GROUP II)	Yellow or pale yellowish brown. 3 - 4 mm in diameter. Yellow agar.
<i>V. alginolyticus</i>	Yellow, large.
<i>Enterobacteriaceae</i>	Scanty growth, punctiform, transparent. No color change in agar.
<i>Pseudomonas</i> , <i>Aeromonas</i>	Blue, small, punctiform.
<i>Enterococcus</i>	Scanty growth, punctiform. Yellow agar.

Microbiological Tests:

Microorganisms	Growth	Medium color
<i>Vibrio cholerae Inaba</i>	Satisfactory	Yellow
<i>Vibrio cholera Ogawa</i>	Satisfactory	Yellow
<i>Vibrio alginolyticus</i>	Moderate	Yellow
<i>Vibrio parathemolyticus ATCC 17802</i>	Satisfactory	Blue
<i>Enterobacter cloacae ATCC 13047</i>	Light	Yellow
<i>Proteus mirabilis ATCC 14273</i>	Moderate	Light blue
<i>Escherichia coli ATCC 25922</i>	Negative	-
<i>Pseudomonas aeruginosa ATCC 27853</i>	Negative-Light	Blue

