

86348 TCBS Agar (Vibrio Selective Agar, Thiosulfate Citrate Bile Sucrose Agar)

Selective agar for the isolation and cultivation of *Vibrio cholera* and other *Vibrio* species.

Composition:

Ingredients	Grams/Litre
Mixed peptone	10.0
Yeast extract	5.0
Sucrose	20.0
Sodium citrate	10.0
Ferric citrate	1.0
Sodium chloride	10.0
Sodium thiosulfate	10.0
Oxbile	5.0
Sodium cholate	3.0
Thymol blue	0.04
Bromothymol blue	0.04
Agar	14.0

Final pH 8.6 +/- 0.2 at 25°C

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Directions:

Dissolve 88 g in 1 litre distilled water and bring to boiling. Pour plates. Do NOT autoclave.

Principle and Interpretation:

This medium is recommended for the isolation and rapid growth of *Vibrio* spp. A negative reaction is no color change after 48 h of incubation from stool specimens and for food testing. The contaminating non-vibrios are suppressed.

TCBS Agar is highly selective, meets the nutritional requirements of *Vibrio* spp., and allows vibrios to compete with intestinal flora. All members of the genus are able to grow in media containing increased salt concentrations and some species are halophilic. Vibrios are natural inhabitants of sea water.

Yeast Extract and mixed peptone provide nitrogenous compounds, vitamin B complex, amino acids and other essential growth nutrients. Oxbile and sodium citrate inhibit gram-positive bacteria like enterococci. Sodium thiosulfate serves as a good source of sulphur, which in combination with ferric citrate detects the production of hydrogen sulfide. For the metabolism of Vibrios, sucrose is added as a fermentable carbohydrate. For the Vibrios, sucrose is a fermentable carbohydrate, and sodium chloride stimulates growth. The mixed indicator bromothymol blue and thymol blue changes its colour to yellow, when acid is formed, even in this strongly alkaline medium. The alkaline pH of the medium improves the recovery of *Vibrio cholerae*.

Strains of *Vibrio cholerae* produce yellow colonies on TCBS Agar because of fermentation of sucrose. *Vibrio alginolyticus* also produce yellow colonies. It's possible that a few sucrose-positive *Proteus* strains can grow to form yellow, vibrid-like colonies. *Vibrio parahaemolyticus* is a sucrose non-fermenting organism and produces blue-green colonies, as does *Vibrio vulnificus*. As mentioned previously, occasional isolates of *Pseudomonas* and *Aeromonas* species also produce blue-green colonies, but overall TCBS Agar is highly selective and any H₂S-negative colony is possibly *Vibrio* species.



The medium should be inoculated heavily with faecal specimens because some *Vibrio* species readily die off on the medium, owing to fermentation of sucrose and accumulation of acids.

Cultural characteristics after 18-24 hours at 35°C.

Organisms (ATCC/WDCM)	Inoculum [CFU]	Growth	Colony Color
<i>Vibrio cholerae</i> (15748/-)	50-100	+++	yellow
<i>Vibrio fluvialis</i> (33809/00137)	50-100	+++	yellow
<i>Vibrio parahaemolyticus</i> (17802/00037)	50-100	+++	bluish green
<i>Vibrio parahaemolyticus</i> (17802/00185)	50-100	+++	green
<i>Vibrio furnissii</i> (-/00186)	50-100	+++	green
<i>Vibrio vulnificus</i> (27562/-)	50-100	+ / ++	greenish yellow
<i>Escherichia coli</i> (25922/00013)	≥10 ⁴	-	-
<i>Proteus mirabilis</i> (14273/-)	≥10 ⁴	- or - / +	can may form yellow colonies
<i>Enterococcus faecalis</i> (29212/00087)	≥10 ⁴	-	-

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