TCBS/Agar
(Thiosulfate - Citrate - Bile - Saccharose)

355-5933

DEFINITION
Medium used to detect and enumerate *Vibrio parahaemolyticus* in shellfish waters and in live seawater shellfish.

STANDARDS
FOOD MICROBIOLOGY
- **NF ISO 8914 (May 1991):** Microbiology - General guidelines for the detection of *Vibrio parahaemolyticus*.

- **Analytical bacteriological methods for the testing of shellfish** (Circular DGAL/SVHA/ C88/ N°8003 on 28th April 1988).

PRINCIPLE
The principle of the medium relies on the inability of *Vibrio parahaemolyticus* to ferment saccharose. Due to the presence of bovine bile and high concentrations of thiosulfate and sodium citrate, the medium inhibits other bacteria.

PRESENTATION
- **Ready-to-use**
  100 ml x 6 bottles **code 355-5933**

- **Dehydrated**
  500 g **code 356-4654**

STORAGE
- Ready-to-use: + 2°C to 8 °C.
- Dehydrated: +15°C to 25°C, in carefully-sealed bottles in a cool, dry place.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Peptone	10 g
Yeast extract	5 g
Sodium citrate	10 g
Sodium thiosulfate	10 g
Sodium chloride	10 g
Bovine bile	8 g
Ferric citrate	1 g
Saccharose	20 g
Bromothymol blue	0.04 g
Thymol blue	0.04 g
Agar	14 g
Distilled water	1,000 ml

Final pH (25°C) = 8.6 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)
- Distilled water

EQUIPMENT REQUIRED (NOT SUPPLIED)
(non-exhaustive)
- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Mixer-homogenizer
- 100 ml Pyrex bottles with autoclave-proof stoppers
- Sterile Petri dishes (Ø = 90 mm)
- Sterile pipettes (**code 355-0751**) or inoculating loops
- Water-bath, precise to ± 1°C
- Thermostatically controlled incubators or incubation room, precise to ± 1°C
- Autoclave
- All usual laboratory equipment.

PROTOCOL
• Preparation of samples
According to the standards applicable to the product concerned.

• Enrichment in selective medium
According to the standards applicable to the product concerned.

• Inoculation and incubation
After incubation of the enrichment medium, isolate on TCBS medium. Incubate 18 h to 24 h at 37°C ± 1°C.

READING AND INTERPRETATION
Reading consists in the observation of the coloration and diameter of the colonies:
- *V. parahaemolyticus*: blue-green colonies, Ø 3 to 5 mm.
- *V. alginolyticus*: yellow colonies, Ø 3 to 5 mm.
- *Enterococcus sp*: yellow colonies, Ø 1 mm.
- *Proteus sp:* yellow-green colonies, Ø 1 mm.
- *Pseudomonas sp*: blue-green colonies, Ø 1 mm.

The identification of various species of *Vibrios* on TCBS is presumptive and must be confirmed by further tests.

Bio-Rad, 3 boulevard Raymond Poincaré • 92430 Marnes-la-Coquette
Tel. : +33 (0)1 47 95 60 00 • Fax : +33 (0)1 47 41 91 33
www.foodscience.bio-rad.fr

N.B.:

1. *E. coli, S. Typhi, Klebsiella, Shigella, P. aeruginosa, Proteus,* likely to grow on this medium, do not present any yellow coloration.

2. Isolated *vibrio* strains should be sent to:

Centre National de Référence des Vibrions
Institut Pasteur
75015 PARIS - FRANCE
Tel: +33.(0)1.43.06.19.19

PRECAUTIONS

- The time lapse between the end of preparation of the stock solution (or the 10^{-1} dilution in the case of a solid product) and the moment when the dilutions come into contact with the culture medium must not exceed 15 minutes.
- Most bacteria other than *Vibrio* are inhibited on this medium for at least 24 hours. Some colonies of *Proteus* and of *Streptococcus faecalis*, however, may appear but they are easily recognisable compared to *Vibrio* colonies.
- Comply with Good Laboratory Practice.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Results after 24-48h culture at 37°C
Vibrio cholerae	Good growth Yellow colonies
Escherichia coli ATCC 25922	Very slight growth or none at all
Vibrio parahaemolyticus CIP 75.2	Good growth Blue-green colonies

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product.
Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

TCBS / *Vibrio parahaemolyticus* / Seawater shellfish / Shellfish waters / Detection / Enumeration / Medium.

BIBLIOGRAPHY

DODIN A. (1982, 10-11 November): Diagnostic du vibrion cholérique au laboratoire. Réunion internationale sur les infections intestinales. Grenada (Spain).

Bio-Rad, 3 boulevard Raymond Poincaré • 92430 Marnes-la-Coquette
Tel. : +33 (0)1 47 95 60 00 • Fax : +33 (0)1 47 41 91 33
www.foodscience.bio-rad.fr