# Technical Sheet

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## **TCBS KOBAYASHI AGAR**

Ready to use flasks



TCBS Agar: Vibrio parahaemolyticus

**INTENDED USE**: ready to use selective plating medium for the isolation of *Vibrio* spp.

10.00
5.00
10.00
10.00
10.00
8.00
20.00
1.00
0.04
0.04
16.00

#### DIRECTIONS

Dissolve the contents of the bottle in a temperature controlled water batch (100°C). Cool to 45-50°C, mix well and distribute into sterile Petri dishes Final pH 8.6  $\pm$  0.2

#### DESCRIPTION

TCBS Kobayashi Agar is prepared according to the original formula of Kobayashi, Enomoto, Sakasaki and Kuwahara. TCBS Agar is recommended by ISO 8914 and by AOAC for the isolation of *Vibrio parahaemolyticus* and *Vibrio cholerae*. The medium because of its alkaline pH, salt content and presence of bile salts, inhibits the growth of most microorganisms except for the halophiles. Enteric bacteria do not grow on TCBS Agar. The rare colonies of some strains of *Proteus* and enterococci are easily distinguished by their reduced size and absence of colour. Sucrose serves as a fermentable carbohydrate that, with the help of bromothymol blue and thymol blue indicators, allows the differentiation of *Vibrio* spp., which utilise sucrose.

#### TECHNIQUE

For the isolation of *V.cholerae* from faeces and from other materials, streak the surface of the plates directly with the material under examination and with the enriched sample in Alkaline Peptone Water (REF 401032), and incubate for 18-24 hours at 35-37°C. Cultures grown on TCBS Agar should be examined immediately after removal from the incubator as yellow colonies of sucrose fermenting vibrios may revert to a green colour when left at room temperature.

*V.cholerae* and its biotype Eltor, after 18-24 hours of incubation, grow on TCBS Agar forming yellow colonies (fermentation of sucrose).

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*Vibrio parahaemolyticus* does not ferment sucrose and grow on TCBS Agar with blue-green colonies. *Vibrio alginolyticus* ferments sucrose and grows with yellow colonies.

The suspected colonies are submitted to oxidase test to string test with sodium desoxycholate and transferred to a Kligler Iron Agar. After subculture on Tryptic Soy Agar the agglutination tests with specific antisera are carried out.

#### USER QUALITY ASSURANCE (37°C-24HRS)

Productivity control *V.fluvialis NCTC 11212*: growth, yellow colonies Selectivity control *E.coli* ATCC 25922: inhibited

#### STORAGE

Store at 2-8° - When stored as directed the medium remains stable until the expiry date shown on the label. Do not use beyond stated expiry date. User prepared plates: 14 days at 2-8°C

#### REFERENCES

• AOAC (1995) - Official Method of Analysis, 16th Ed.

• ISO 8914 - Microbiology - General Guidance for the detection of Vibrio parahaemolyticus. 1990-12-01

• FDA (1995)Bacteriological Analytical Manual, 8<sup>th</sup> ed. Revision A, 1998. Published by AOAC International.

• Kobayashi, T., Enomoto, S., Sakasaki, R. & Kuwahara S.(1963) Jap. J.Bacteriol., 18, 10-11; 392-397.

• MacCormack, W.M., et al., E.J. (1974) -. J. Inf. Dis., 129, 497-500.

### PACKAGING 5121062 TCBS Agar 6 x 100ml ready to use flasks