

TCBS Agar

Intended use

TCBS Agar is a selective medium used for isolating and cultivating *Vibrios* causing cholera and food poisoning from clinical and non-clinical specimens.

Summary

Thiosulphate Citrate Bile Salts Sucrose (TCBS) Agar is a primary plating medium for the selective isolation of *Vibrios* that cause cholera, diarrhoea and food poisoning. It was developed by Nakanishi *et al.*, and was modified by Kobayashi. The combination of alkaline peptone water and TCBS Agar is used in many procedures for the isolation of *V. cholerae* and other *Vibrio* species from foods, water and faeces. This medium is included in the Bacteriological Analytical Manual for food testing.

Principle

Proteose peptone and yeast extract provide nitrogenous compounds, vitamin B complex and other essential growth nutrients. Gall powder and sodium citrate inhibits Gram-positive bacteria. Sodium thiosulphate serves as the sulphur source and in combination with ferric citrate detects hydrogen sulphide production. Sucrose is the fermentable carbohydrate for the metabolism of *Vibrios*. The alkaline pH of the medium enhances the recovery of *V. cholerae*. Thymol blue and bromothymol blue are included as indicators of pH change. Sodium chloride maintains the osmotic balance.

Formula*

Ingredients	g/L
Sucrose	20.0
Proteose Peptone	10.0
Sodium Citrate	10.0
Sodium Thiosulphate	10.0
Sodium Chloride	10.0
Gall Powder [#]	8.0
Yeast Extract	5.0
Ferric Citrate	1.0
Thymol Blue	0.04
Bromothymol Blue	0.04
Agar	15.0
Final pH (at 25°C)	8.6 ± 0.2

*Adjusted to suit performance parameters.

[#]Equivalent to intended performance of Oxygall

Storage and Stability

Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen

Clinical samples; Food samples; Water sample

Specimen Collection and Handling

Ensure that all samples are properly labelled.

Follow appropriate techniques for handling samples as per established guidelines.

Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.

The samples must be stored and tested within the permissible time duration.

After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 89.08 g of the powder in 1000 mL purified / distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. DO NOT AUTOCLAVE.
5. Cool to 45°C-50°C and pour into sterile petridishes, Use immediately.

Quality Control

Dehydrated Appearance: Cream to yellow coloured with tan cast, homogeneous, free flowing powder.

Prepared Appearance: Green coloured, slightly opalescent gel forms in petridishes.

Cultural Response: Cultural characteristics observed after an incubation of 18-24 hours at 30°C-35°C.

Organism (ATCC)	Growth	Colour of Colony
<i>Vibrio cholerae</i> (14748)	Good	Yellow
<i>Vibrio parahaemolyticus</i> (MTCC 451)	Good	Blue green
<i>Escherichia coli</i> (25922)	Complete Inhibition	-
<i>Proteus hauseri</i> (13315)	Partial Inhibition	Yellow
<i>Streptococcus faecalis</i> (11420)	Partial Inhibition	Yellow

Interpretation of Results

Colony morphology on TCBS Agar is as follows:

V. cholerae -----Yellow colonies

V. parahaemolyticus -----Colonies with blue to green centers

Proteus/Enterococcus ----- Partial inhibition. If growth occurs, colonies are small, yellow and translucent.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. Cultures grown on TCBS Agar should be examined immediately after removal from the incubator as yellow colonies of *Vibrios*, e.g. *V. cholerae* may revert to a green colour when left at room temperature.
2. The identification of the various *Vibrio* species on TCBS Agar is presumptive and further tests are required for confirmation.
3. Yellow colonies on TCBS Agar will give unsatisfactory oxidase reactions.
4. Initial isolation of *V. parahaemolyticus* may be confused with *Plesiomonas shigelloids* and *Pseudomonas* species.
5. Sucrose fermenting *Proteus* species produce yellow colonies, which may resemble those of *Vibrio*.
6. A few strains of *V. cholerae* may appear green or colourless on TCBS Agar due to delayed.
7. Colonies taken from TCBS Agar are 'sticky' and react poorly in slide agglutination tests. Subculture to Nutrient Agar before slide agglutination tests are carried out.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

References

1. Kobayashi T., Enomoto S., Sakazaki R., and Kuwahara S., 1963, Jap. J. Bacteriol., 18: 387.
2. Nakanishi Y., 1963, Modern Media 9: 246.
3. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
4. Clesceri L. S., Greenberg A. E. and Eaton A. D., (Eds.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., American Public Health Association, Washington, D.C.
5. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201200010100	Dehydrated Culture Media	100 g
201200010500	Dehydrated Culture Media	500 g

 Temperature Limit	 Manufacturer	 LOT	Batch Code	 Date of Manufacture	 This way up	 Received on
 REF Catalogue Number	 Consult Instructions for use	 Use-by Date	 Hygroscopic keep container tightly closed	 OO Opened on		

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Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.