TCBS AGAR

INTENDED USE
Remel TCBS Agar is a solid medium recommended for use in qualitative procedures for selective isolation of *Vibrio cholerae* and other enteropathogenic *Vibrio* spp. from clinical and non-clinical specimens.

SUMMARY AND EXPLANATION
Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS) was developed by Nakanishi and modified by Kobayashi et al. in 1963.[1,2] It was developed for selective isolation of vibrios which cause cholera, diarrhea, and food poisoning. TCBS Agar is recommended by the World Health Organization (WHO) for isolation of *Vibrio cholera*.[3] In 1982, West et al. reported TCBS Agar could be used for recovery of certain new pathogens such as *Vibrio fluvialis* and *Vibrio vulnificus*.[4] TCBS Agar is also recommended by the AOAC International (AOAC).[5]

PRINCIPLE
Casein and meat peptones supply essential nitrogenous substances, amino acids, and vitamins necessary for bacterial growth. Yeast extract is a growth enhancer. Oxgall, which contains a mixture of bile salts, is a selective agent which inhibits gram-positive organisms. Coliforms, *Proteus*, *Pseudomonas*, and *Aeromonas* are partially inhibited. Sodium thiosulfate, in combination with ferric citrate, detects hydrogen sulfide production. Sucrose serves as the carbohydrate fermented by *Vibrio* spp. Brom thymol blue and thymol blue make up the pH indicator system. TCBS Agar has an elevated pH to enhance the recovery of *V. cholerae* which is sensitive to a low pH.

REAGENTS (CLASSICAL FORMULA)*

Sucrose	20.0 g	Yeast Extract	5.0 g
Sodium Chloride	10.0 g	Sodium Cholate	3.0 g
Sodium Citrate	10.0 g	Ferric Citrate	1.0 g
Sodium Thiosulfate	10.0 g	Brom Thymol Blue	0.04 g
Casein Peptone	5.0 g	Thymol Blue	0.04 g
Meat Peptone	5.0 g	Agar	15.0 g
Oxgall	5.0 g	Demineralized Water	1000.0 ml

pH 8.6 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PRECAUTIONS
This product is For Laboratory Use only. It is not intended for use in the diagnosis of disease or other conditions.

PREPARATION OF DEHYDRATED CULTURE MEDIUM
1. Suspend 89 g of medium in 1000 ml of demineralized water.
2. Heat to boiling with agitation to completely dissolve. **Do not autoclave**.
3. Cool to 45-50°C and dispense into appropriate containers.

PROCEDURE
1. Consult current editions of appropriate references for the recommended procedure for sample preparation, inoculation, testing, and interpretation.

QUALITY CONTROL
Each lot number of TCBS Agar has been manufactured, packaged, and processed in accordance with current Good Manufacturing Practice regulations. All lot numbers have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

CONTROL	INCUBATION	RESULTS
Vibrio alginolyticus ATCC®17749	Ambient, 18-24 h @ 33-37°C	Growth, yellow colonies
Vibrio parahaemolyticus ATCC®17802	Ambient, 18-24 h @ 33-37°C	Growth, green colonies
Escherichia coli ATCC®25922	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)
Salmonella enterica serovar Typhimurium ATCC®14028	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)
Shigella flexneri ATCC®12022	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)

LIMITATIONS
1. A few strains of *V. cholerae* may appear green or colorless on TCBS Agar due to delayed sucrose fermentation.[6]
2. TCBS Agar is an unsatisfactory medium for oxidase testing and direct serological agglutination of isolated microorganisms.[6,7]
3. Colonies that resemble *Vibrio* spp. on TCBS Agar require further biochemical and/or serological testing for identification.[8]

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, sample collection, storage and transportation, materials required, quality control, and limitations.

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