



BD™ TCBS Agar

INTENDED USE

BD TCBS Agar (Thiosulfate-Citrate-Bile-Sucrose Agar) is a selective differential medium for isolating and cultivating *Vibrio cholerae* and other *Vibrio* species from clinical specimens and other materials.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Vibrios are natural inhabitants of brackish and salt water worldwide.^{1,2} Human intestinal disease has been associated with consumption of contaminated water and shellfish or other seafood. *Vibrio cholerae* is the etiologic agent of a secretory diarrhea (cholera), spread by the ingestion of contaminated drinking water and foods and by the fecal-oral route.² Several other *Vibrio* species, e.g., *V. parahaemolyticus* and *V. fluvialis*, are the cause of acute gastroenteritis. Additionally, several *Vibrio* species, e.g. *V. alginolyticus*, *V. vulnificus*, and *V. damsela*, are associated with extraintestinal infections, such as wound infections, septicemia, meningitis, and others.¹⁻³ Wound infections with *Vibrio* have been shown to occur especially if patients had contact with salt and brackish water.^{2,3}

BD TCBS Agar, prepared according to the formula of Kobayashi *et al.*, is a modification of the selective medium from Nakanishi.^{4,5} All *Vibrio* spp. that are pathogenic to humans, except *V. hollisae*, will grow on this medium. This medium is recommended for isolating *Vibrio* spp. from stool specimens^{1,2,6} and is mentioned in Standard Methods for food testing.^{7,8} It 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.⁶

In **BD TCBS Agar**, yeast extract and peptone provide the nitrogen and vitamins. Sodium citrate, sodium thiosulfate, oxgall, and cholate are selective agents which provide an alkaline pH to inhibit gram-positive organisms and suppress coliforms. The pH of the medium is increased to enhance growth of *Vibrio cholerae* because this organism is sensitive to acid environments. The high sodium concentration favors growth of *Vibrio cholerae* which is halotolerant and of other *Vibrio* species most of which are halophilic. Sucrose is a fermentable carbohydrate, and sodium chloride stimulates growth. Sodium thiosulfate is a sulfur source and acts with ferric citrate as an indicator to detect hydrogen sulfide production. Bromthymol blue and thymol blue are pH indicators.

REAGENTS

BD TCBS Agar

Formula* Per Liter Purified Water

Yeast Extract	5.0 g	Saccharose (Sucrose)	20.0 g
Pancreatic Digest of Casein	5.0	Sodium Chloride	10.0
Peptic Digest of Animal Tissue	5.0	Ferric Citrate	1.0
Sodium Citrate	10.0	Brom Thymol Blue	0.04
Sodium Thiosulfate	10.0	Thymol Blue	0.04
Oxgall	5.0	Agar	14.0
Sodium Cholate	3.0		

pH 8.6 ± 0.2

*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate for 18 to 24 hours aerobically at 35 to 37° C.

Strains	Growth Results
<i>V. cholerae</i> NCTC 8021 or ATCC 9459	Growth fair to excellent; yellow zones surrounding colonies
<i>V. parahaemolyticus</i> ATCC™ 17802	Growth fair to excellent; green to blue-green colonies; medium nearly unchanged
<i>V. alginolyticus</i> ATCC 17749	Growth fair to excellent; yellow zones surrounding colonies
<i>E. faecalis</i> ATCC 29212	Inhibition partial to complete; small yellow colonies
<i>E. coli</i> ATCC 25922	Inhibition partial to complete; colonies small, translucent
<i>Ps. aeruginosa</i> ATCC 27853	Inhibition partial to complete; blue colonies
Uninoculated	Green to blue-green

PROCEDURE

Materials Provided

BD TCBS Agar (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types and Transport of Specimens

This medium is used for the isolation of *Vibrio* species from stool specimens (especially if patients have consumed seafood) or from extraintestinal clinical specimens if *Vibrio* species are suspected (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**). Specimens such as rectal swabs, vomitus and food samples, especially seafood, may also be used. Swabs should be transported in Cary and Blair transport medium because *Vibrio* spp. are particularly susceptible to drying.² All intestinal specimens and food samples may be placed in tubes with alkaline peptone water to prevent desiccation of the materials and should be transported to the laboratory without delay. Transport times of more than 8 hours will reduce the viability. Do not freeze the specimens or samples!

Test Procedure

Streak the specimen onto **BD TCBS Agar** directly after its arrival in the laboratory, using an approved streaking technique. Specimens and food samples may be swabbed directly or after careful homogenization (especially when seafood is tested). Avoid desiccation during handling. It is recommended to include a less selective medium such as **BD DCLS Agar** which is superior to MacConkey Agar in isolating *Vibrio* species. All extraintestinal specimens (e.g. specimens from wounds etc.) must also be plated onto a nonselective blood agar medium, e.g., **BD Columbia Agar with 5% Sheep Blood**, and on a **BD MacConkey II Agar** plate, to provide detection of other pathogens possibly involved in the infection. Incubate plates aerobically at 35 to 37 ° C for 18 to 24 hours. If negative, incubate for an additional 24 hours.

Samples and clinical specimens with suspected low counts of *Vibrio* can first be enriched by incubating an aliquot of the specimen or sample in alkaline peptone water at $35 \pm 2^\circ \text{C}$.^{2,8} Subcultures are made on TCBS and blood agar after 8 and again after 18 hours of incubation.

Results

On **BD TCBS Agar**, sucrose-fermenting vibrios (*V. cholerae*, *V. alginolyticus*, *V. harveyi*, *V. cincinnatiensis*, *V. fluvialis*, *V. furnissii*, *V. metschnikovii*) appear as medium-sized, smooth, opaque, yellow colonies. Most other clinically important vibrios, including *V. parahaemolyticus*, do not ferment sucrose and appear as green colonies.² Additional biochemical and/or biochemical tests are necessary for a final identification and for a differentiation of sucrose-fermenting and sucrose-nonfermenting species.^{1,2,7,8}

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD TCBS Agar is a standard medium for the isolation of *Vibrio cholera* or other *Vibrio* species from stool specimens of patients suffering from diarrhea, especially after seafood consumption, or if outbreaks of cholera are suspected.^{1,2,6,7} It may also be used for detecting *Vibrio* in extraintestinal specimens.

BD TCBS Agar is also a standard medium for the isolation of *Vibrio* from foods.^{8,9}

Since the nutritional requirements of organisms vary, some strains may grow poorly on this medium. Therefore, less selective media which allow the detection of *Vibrio* species and of other intestinal pathogens (e.g., *Salmonella* and *Shigella*) should also be included; **BD DCLS Agar** can be recommended for this purpose.

On **BD TCBS Agar**, *V. parahaemolyticus* may resemble *Aeromonas hydrophila*, *Plesiomonas shigelloides* and *Pseudomonas* species. Sucrose-fermenting *Proteus* species produce yellow colonies which may resemble those of *Vibrio*. A few strains of *V. cholerae* appear green or colorless on **BD TCBS Agar** due to delayed sucrose fermentation.

TCBS media are unsatisfactory for oxidase testing of *Vibrio* spp.²

Further tests are necessary for complete identification and confirmation of *Vibrio* spp. isolated on this medium. Consult the references.^{2,6-9}

REFERENCES

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PACKAGING/AVAILABILITY

BD TCBS Agar

Cat. No. 254432

Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

For further information please contact your local BD representative.



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