QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION

BD BBL™ Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS) is recommended for use in the selective isolation of vibrios.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Streak the plates for isolation. Use cultures diluted to yield 10^3–10^5 CFU/plate.
   b. Incubate plates at 35 ± 2 °C in an aerobic atmosphere.
   c. Include **BD Trypticase** Soy Agar with 5% Sheep Blood plates as nonselective controls for all organisms.
2. Examine plates after 18–24 h for amount of growth, pigmentation, colony size and selectivity.
3. Expected Results

Organisms	ATCC®	Recovery	Colony Color
**Vibrio cholerae*	9459	Fair to heavy growth	Yellow
**Vibrio parahaemolyticus*	17802	Fair to heavy growth	Blue
**Escherichia coli*	25922	Partial or complete inhibition	If growth, colonies are small and translucent
**Pseudomonas aeruginosa*	10145	Partial or complete inhibition	If growth, colonies are blue
**Enterococcus faecalis*	29212	Partial or complete inhibition	If growth, colonies are small and yellow

*Recommended organism strain for User Quality Control.

NOTE: This medium is exempt from User Quality Control according to CLSI M22-A3.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 8.9 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 30 ± 1 °C for 60 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

BD BBL Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS) is used for the selective isolation of cholera vibrios and *Vibrio parahaemolyticus* from a variety of clinical and nonclinical specimens.

V SUMMARY AND EXPLANATION

Vibrio species are most widely recognized for their role in human intestinal infections, and cholera and *V. parahaemolyticus* diarrhea are important worldwide. The isolation and cultivation of *Vibrio* species has been enhanced by the development of media which are highly selective for vibrios.

BD BBL TCBS Agar is the primary plating medium universally used for the selective isolation of vibrios causing cholera, diarrhea and food poisoning. It was developed by Kobayashi et al.[1], who modified the selective medium of Nakanishi.[2] The combination of alkaline peptone water and **BD BBL** TCBS Agar is used in many procedures for the isolation of *V. cholerae* and other *Vibrio* species from feces.[3-5]

VI PRINCIPLES OF THE PROCEDURE

BD BBL TCBS Agar is highly selective for the isolation of *V. cholerae* and *V. parahaemolyticus* as well as other vibrios. Inhibition of gram-positive bacteria is achieved by the incorporation of oxgall, a naturally occurring substance containing a mixture of bile salts, and sodium cholate, a pure bile salt. Sodium thiosulfate serves as a sulfur source and, in combination with ferric citrate, detects hydrogen sulfide production. Sucrose is included as a fermentable carbohydrate for the metabolism of vibrios. The alkaline pH of the medium enhances the recovery of *V. cholerae*. Thymol blue and bromthymol blue are included as indicators of pH changes.

VII REAGENTS

BD BBL TCBS Agar

Approximate Formula* Per Liter Purified Water

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

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

Warnings and Precautions: For *in vitro* Diagnostic Use in Singapore.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"[6-9] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8 °C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

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

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[10,11] Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: BD BBL TCBS Agar

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Streak the specimen as soon as possible after it is received in the laboratory. Specimens such as rectal swabs, feces, vomitus, fish or food samples may be swabbed directly onto the plated medium. Heavy inoculation is recommended, especially if specimens are not fresh, as the medium is highly selective and vibrios tend to die rather easily. Swabs containing specimen material should be transported to the laboratory in Cary and Blair Transport Medium[3,12] if a delay in reaching the laboratory is anticipated. Specimens for cultivation of vibrios should not be frozen.

Incubate plates, protected from light, at 35 ± 2 °C in an aerobic atmosphere for 18–24 h.

User Quality Control:

Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory's standard quality control procedures.

X RESULTS

After incubation, most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Better isolation is obtained due to the inhibitory action of the medium.

Typical colonial morphology on **BD BBL** TCBS Agar is as follows:

V. cholerae ..... Large yellow colonies
V. parahaemolyticus ..... Colonies with blue to green centers
V. alginolyticus ..... Large yellow colonies
Proteus/Enterococci ..... Partial inhibition. If growth, colonies are small and yellow to translucent.
Pseudomonas/Aeromonas ..... Partial inhibition. If growth, colonies are blue.

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[10,11,13-16]

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII AVAILABILITY

Cat. No.	Description
251143	**BD BBL**™ TCBS Agar, Pkg. of 20 plates
251137	**BD BBL**™ TCBS Agar, Ctn. of 100 plates

XIII REFERENCES

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