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Instructions for Use

# TCBS (THIOSULFATE CITRATE BILE SALTS SUCROSE) AGAR

| | | |
|---|---|---|
| Cat. no. G55 | TCBS Agar, 15x100mm Plate, 18ml | 10 plates/bag |

## INTENDED USE

Hardy Diagnostics TCBS Agar (Thiosulfate Citrate Bile Salts Sucrose Agar) is recommended for the selective isolation and cultivation of *Vibrio* spp. from clinical specimens.

## SUMMARY

TCBS Agar is prepared according to the formula developed by Kobayashi, et al.[6] It is highly selective for the isolation of *V. cholerae* and *V. parahaemolyticus* , in addition to other *Vibrio* spp. TCBS has a very high pH (8.5-9.5) which suppresses growth of intestinal flora other than *Vibrio* spp.[5] The medium consists of plant and animal proteins, a mixture of bile salts, one percent sodium chloride, sodium thiosulfate, ferric citrate, sucrose, and yeast extract. The bile salts inhibit growth of gram-positive microorganisms; one percent sodium chloride is incorporated into the medium to provide optimum growth and metabolic activity of halophilic *Vibrio* spp.; sodium thiosulfate provides a source of sulfur and also acts in combination with ferric citrate to detect the production of hydrogen sulfide; sucrose serves as the fermentable carbohydrate that, with the help of bromothymol blue and thymol blue indicators, allows for the differentiation of those *Vibrio* spp. which utilize sucrose.

*V. cholerae* and its biotype Eltor ferment sucrose, which results in a pH shift and production of yellow-brown colonies. According to Fishbein, et al., *V. parahaemolyticus* will produce light bluish colonies.[7] Certain strains of *Proteus* and enterococci may grow and produce small, yellow colonies that are easily distinguished.

*Vibrio* species that are considered medically important can be divided into two groups, *V. cholerae* and the non-cholera *Vibrio* spp.[5] They are as follows:

| ***V. cholerae* :** | **Non-cholera *Vibrio* spp.:** |
|---|---|
| *V. cholerae* serogroup O1 | *V. alginolyticus* |
| *V. cholerae* serogroup non-O1 | *V. carchariae* |
| | *V. cincinnatiensis* |
| | *V. damsela* |
| | *V. fluvialis* |
| | *V. furnissii* |
| | *V. hollisae* |
| | *V. metschnikovii* |

| | |
|---|---|
| | *V. mimicus* |
| | *V. parahaemolyticus* |
| | *V. vulnificus* (lactose-fermenter) |

*Vibrio cholerae* is the causative agent of cholera. Other *Vibrio* species have been associated with gastroenteritis and extraintestinal infections, especially of the ear, soft tissue, and blood. Life-threatening septicemia has been linked to *V. vulnificus* . Most *Vibrio* infections are associated with seawater contact. Symptoms are often similar to more common inland microbial agents.

## FORMULA

Ingredients per liter of deionized water:*

| | |
|---|---|
| Sucrose | 20.0gm |
| Dipeptone | 10.0gm |
| Sodium Citrate | 10.0gm |
| Sodium Thiosulfate | 10.0gm |
| Sodium Chloride | 10.0gm |
| Yeast Extract | 5.0gm |
| Oxbile (Oxgall) | 5.0gm |
| Sodium Cholate | 3.0gm |
| Ferric Citrate | 1.0gm |
| Bromothymol Blue | 0.04gm |
| Thymol Blue | 0.04gm |
| Agar | 15.0gm |

Final pH 8.6 +/- 0.2 at 25ºC.

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

## STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

## PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not

ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

## PROCEDURE

Specimen Collection: Infectious material should be submitted directly to the laboratory within two to three hours of collection. Specimens should be protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport medium and refrigerated until inoculation. Consult listed references for information on specimen collection.[1-5]

Method of Use: Media should be warmed to room temperature prior to inoculation. The specimen, or sampling from a well mixed transport medium, should be inoculated directly onto the TCBS Agar and streaked so as to obtain isolated colonies. Incubate aerobically at 35ºC. for 18-24 hours. Examine for characteristic colonial morphology. Cultures grown on TCBS should be examined immediately after removal from the incubator as yellow colonies of *Vibrio* spp. (e.g., *V. cholerae* ) may revert to a green color when left at room temperature.[8] Consult listed reference for additional information regarding typical colonial morphology.[2,3,5]

## INTERPRETATION OF RESULTS

*V. cholerae* and its biotype Eltor ferment sucrose, which results in a pH shift and production of yellow-brown colonies. According to Fishbein, et al., *V. parahaemolyticus* will produce light bluish colonies.[7] Certain strains of *Proteus* and enterococci may grow and produce small, yellow colonies that are easily distinguished.

| **Typical colonial morphology on TCBS Agar:** | |
|---|---|
| *V. cholerae* | Yellow colonies. |
| *V. parahaemolyticus* | Blue to green centered colonies. |
| *V. alginolyticus* , *V. fluvialis* , *V. furnissii* | Yellow colonies. |
| *V. mimicus* , *V. damsela* | Green colonies. |
| *V. vulnificus* | Green (85%) or yellow (15%). |
| *V. hollisae* | Green (very poor growth). |
| *V. metschnikovii* | Yellow (reduced growth). |
| *Proteus* /Enterococci | Partial to complete inhibition. If growth, small, yellow to translucent colonies. |
| *Pseudomonas* / *Aeromonas* | Partial to complete inhibition. If growth, blue colonies. |
| *Escherichia coli* | Partial to complete inhibition. If growth, translucent colonies. |

## LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

TCBS Agar may not support good growth of some *Vibrio* spp. (e.g., *V. hollisae* and *V. metschnikovii* ). The identification of the various *Vibrio* spp. on TCBS Agar is presumptive and further tests are required for confirmation.

It is recommended that a non-selective media be used in conjunction with selective media for optimum recovery of pathogenic organisms.

Cultures grown on TCBS should be examined immediately after removal from the incubator as yellow colonies of *Vibrio* spp. (e.g., *V. cholerae* ) may revert to a green color when left at room temperature.[8]

Colonies that appear yellow on TCBS Agar will produce unsatisfactory oxidase reactions.

If slide agglutination tests are to be carried out, organisms must be subcultured to nutrient agar. Colonies taken from TCBS Agar react poorly in slide agglutination tests due to their 'sticky' nature.

Refer to the document "Limitations of Procedures and Warranty" for more information.

## MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

## QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

| Test Organisms | Inoculation Method* | Incubation | | | Results |
|---|---|---|---|---|---|
| | | Time | Temperature | Atmosphere | |
| *Vibrio parahaemolyticus* ATCC® 17802 | B | 18-24hr | 35°C | Aerobic | Growth; blue-green centered colonies |
| *Escherichia coli* ATCC® 25922 | B | 18-24hr | 35°C | Aerobic | Partial to complete inhibition; small, clear colonies |
| *Proteus mirabilis* ATCC® 12453 | B | 18-24hr | 35°C | Aerobic | Partial to complete inhibition; small, clear to yellow colonies |

* Refer to the document "Inoculation Procedures for Media QC" for more information.

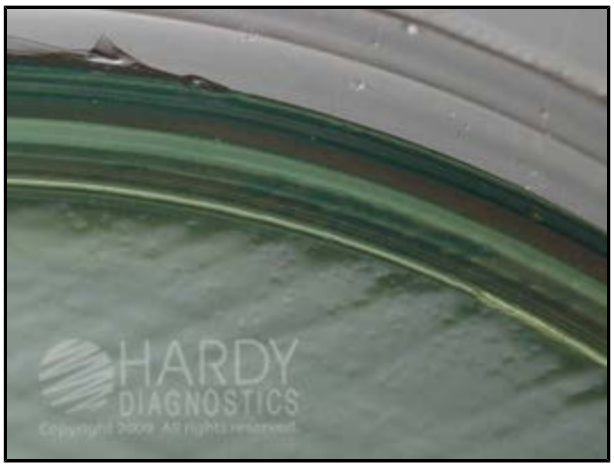
### USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

## PHYSICAL APPEARANCE

TCBS Agar should appear slightly opalescent with no precipitate, and dark green in color.

*Vibrio parahaemolyticus* (ATCC® 17802) colonies growing on TCBS Agar (Cat. no. G55). Incubated aerobically for 24 hours at 35ºC.



*Escherichia coli* (ATCC® 25922) growth inhibited on TCBS Agar (Cat. no. G55). Incubated aerobically for 24 hours at 35ºC.

## REFERENCES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2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

6. Kobayashi, T., et al. 1963. *Jap. J. Bacteriol.*; 18:387.

7. *Applied Microbiology*; 20:176, 1970.

8. Furniss, A.L., et al. 1978. *PHLS Monograph*; No. 11.




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