IFU

Instructions for Use

CRITERION™ TCBS (THIOSULFATE CITRATE BILE SALTS SUCROSE) AGAR

Cat. no. C7040	CRITERION™ TCBS Agar	176gm
Cat. no. C7041	CRITERION™ TCBS Agar	500gm
Cat. no. C7042	CRITERION™ TCBS Agar	2kg
Cat. no. C7043	CRITERION™ TCBS Agar	10kg
Cat. no. C7044	CRITERION™ TCBS Agar	50kg

INTENDED USE

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

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

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 :
V. cholerae serogroup O1
V. cholerae serogroup non O1

V. cincinnatiensis
V. damsela
V. fluvialis
V. furnissii
V. hollisae
V. metschnikovii
V. mimicus
V. parahaemolyticus
V. vulnificus (lactose-fermenter)
Non-cholera *Vibrio* spp.:
V. alginolyticus
V. carchariae

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

Gram weight per liter:	88.0gm/L
Sucrose	20.0gm
Agar	14.0gm
Sodium Chloride	10.0gm
Dipeptone	10.0gm
Sodium Citrate	10.0gm
Sodium Thiosulfate	10.0gm
Oxbile (Oxgall)	5.0gm
Yeast Extract	5.0gm
Sodium Cholate	3.0gm
Ferric Citrate	1.0gm
Bromothymol Blue	0.04gm
Thymol Blue	0.04gm

Final pH 8.6 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original light tan with greenish tinge.

Store the prepared culture media at 2-8ºC.

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 blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory 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.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 89.0gm of the dehydrated culture media in 1 liter of distilled or deionized water.

2. Heat to boiling and mix to dissolve completely.

3. Heat Sensitive. **Do not autoclave.**

4. Cool to 45-50ºC. and dispense into sterile petri dishes or as desired.

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references or refer to the prepared media Instructions for Use (IFU) for Cat. No. G55.

LIMITATIONS

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

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

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.

Sucrose-fermenting *Proteus* species produce yellow colonies which resemble those of *Vibrio* spp.

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

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 color when left at room temperature.[8]

Most *Vibrio* spp. and/or 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 autoclaves, incinerators, and incubators, etc., 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	A	18-24hr	35°'C	Aerobic	Growth; blue-green centered colonies
Escherichia coli ATCC® 25922	B	18-24hr	35°C	Aerobic	Partial to compete 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

Users of dehydrated 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. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION™ TCBS Agar powder should appear homogeneous, free-flowing, and light tan with a greenish tinge in color. The prepared media should appear slightly opalescent with no precipitate, and dark green in color.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

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. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

7. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* AOAC, Arlington, VA. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm.

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

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

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

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1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207B [D]