TECHNICAL DATA SHEET

THIOSULFATE-CITRATE-BILE-SACCHAROSE (TCBS) AGAR

DETECTION AND ISOLATION OF *VIBRIO*

1 INTENDED USE

Thiosulfate-Citrate-Bile-Sucrose Agar is a selective medium for the isolation of *Vibrio cholerae* and other enteropathologic *Vibrio* (in particular *Vibrio parahaemolyticus*) in fish, seafood and biological samples of animal origin.

The typical composition corresponds to that defined in the standard NF EN ISO 21872-1.

2 HISTORY

The original formula developed by Nakanishi was subsequently modified by Kobayashy *et al.* for the selective isolation of pathogenic *Vibrio* species.

3 PRINCIPLES

The high concentrations of thiosulfate and sodium citrate, as well as the alkalinity of the medium, considerably inhibit the growth of enterobacteria.

Ox bile and sodium cholate slow the growth of enterococci and inhibit the development of Gram positive bacteria.

The acidification of the medium resulting from the fermentation of sucrose by *Vibrio* makes bromothymol blue turns yellow.

Using thiosulfate as a sulfur source, the production of hydrogen sulfide is visualized in the presence of ferric citrate. All *Vibrio* are H_2S-negative.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal perforrmance.

For 1 liter of media :

- Polypeptone ..... 10,0 g
- Yeast extract ..... 5,0 g
- Saccharose ..... 20,0 g
- Bacteriological ox bile ..... 5,0 g
- Sodium cholate ..... 3,0 g
- Sodium citrate ..... 10,0 g
- Sodium thiosulfate..... 10,0 g
- Sodium chloride ..... 10,0 g
- Ferric ammonium citrate ..... 1,0 g
- Bromothymol blue ..... 40,0 mg
- Thymol blue..... 40,0 mg
- Bacteriological agar..... 14,0 g

pH of the ready-to-use media at 25 °C : 8,6 ± 0,2.

5 PREPARATION

- Dissolve 88,1 g of dehydrated media (B040) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Do not autoclave.
- Cool and maintain the media in a molten state at 44-47 °C.
- Pour into sterile Petri plates and let solidify on a cold, flat surface.
- Dry the plates in an incubator, covers partially removed.

✓ **Reconstitution :**
88,1 g/L

✓ **Sterilization :**
Bring to boil

6 INSTRUCTIONS FOR USE

- Inoculate and isolate a loop of each enrichment broth onto the surface of agar paltes prepared as above and onto a second media of choice.
- Incubate at 37 ± 1 °C for 24 ± 3 hours.

✓ **Inoculation :**
On surface

✓ **Incubation :**
24 ± 3 h at 37 °C

7 RESULTS

Colonies present the following aspects :

Characteristics	Microorganisms
Flat yellow colonies, 2 to 3 mm in diameter	*Vibrio cholerae* *Vibrio fluvialis,* *Vibrio furnissii*
Flat, green colonies, 2 to 3 mm in diameter	*Vibrio vulnificus* *Vibrio parahaemolyticus* *Vibrio mimicus*
Blue colonies Tiny, transparent colonies	*Pseudomonas, Aeromonas* Enterobacteriaceae or others

8 QUALITY CONTROL

Dehydrated media : greenish-beige powder, free-flowing and homogeneous.
Prepared media : dark green agar.

Typical culture response after 24 hours of incubation at 37 °C (NF EN ISO 11133) :

Microorganisms		Growth	Characteristics
Vibrio furnissii	WDCM 00186	Good	Yellow colonies
Vibrio parahaemolyticus	WDCM 00185	Good	Green colonies
Escherichia coli	WDCM 00013	Inhibited	-

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.
The expiration date is indicated on the label.

Prepared media in vials (*) : Not recommended.
Prepared media in plates (*) : 8 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :
500 g bottle BK040HA

11 BIBLIOGRAPHY

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12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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