PRODUCT SPECIFICATION SHEET

TCBS Agar (DM376BS)

Intended Use
TCBS Agar (DM376BS) is a differential medium recommended for selective isolation of *Vibrio cholerae* and other *enteropathogenic Vibrios*, as per Indian Standard published by BIS.

Product Summary and Explanation
Vibrio spp. Are most widely recognized for their role in causing Cholera and diarrhea diseases and also causing food poisoning. The isolation and cultivation of Vibrio species has been enhanced by the development of media which are highly selective for vibrios. It was developed by Kobayashi et al.[1], who modified the formula of Nakanishi.[2] , which resulted in TCBS Agar, a selective and differential media for the isolation and cultivation of vibrios. Although this medium was originally designed for the isolation of V. Cholera and V. parahaemolyticus , most Vibrios grow to healthy large colonies with many different colonial morphologies. Present formulation of TCBS Agar is recommended by BIS[3] for isolation of *Vibrio cholerae* and *Vibrio parahaemolyticus*. Inoculate the sample in Alkaline Peptone Water (DM009BS) incubate overnight at 35°C. Subculture the growth TCBS Agar.

Principles of the Procedure
Peptone special and yeast extract provide nitrogenous compounds, vitamin B complex and other essential growth Nutrients. TCBS has a very high pH (8.5-9.5) which suppresses growth of intestinal flora other than *Vibrio* spp.[9] Oxbile, a derivative of bile salts and sodium citrate inhibit gram-positive bacteria and coliforms.[10] TCBS Agar, Selective has an additional selective ingredient i.e. sodium cholate for improved selectivity. One percent sodium chloride is incorporated into the medium to provide optimum growth and metabolic activity of halophilic *Vibrio* spp. Sodium thiosulphate serves as a good source of sulphur, which in combination with ferric citrate detects the production of hydrogen sulphide. Sucrose is fermentable sugar, with the help of bromothymol blue and thymol blue indicators, allows for the differentiation of those *Vibrio* spp. which utilize sucrose. The alkaline pH of the medium improves the recovery of *V. cholera* . Strains of *V. cholera* produce yellow colonies on TCBS Agar because of fermentation of sucrose. *V. alginolyticus* also produce yellow colonies. *V. parahaemolyticus* is a sucrose non-fermenting organism and therefore produces blue-green colonies, as does *V. vulnificus* . species that are sucrose-fermenters may form yellow colonies[9]. TCBS Agar is not a suitable medium for oxidase testing of *Vibrio* species.[10] A few strains of *V. cholera* may appear green or colourless on TCBS Agar due to delayed sucrose fermentation.[9]

Formula / Liter

Ingredients	Gms / Litre
Peptone, Special	10.00
Yeast Extract	5.00
Sodium Citrate	10.00
Sodium Thiosulfate	10.00
Oxbile	5.00
Sodium Cholate	3.00
Sucrose	20.00
Sodium Chloride	10.00

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Ferric Citrate	1.00
Bromothymol Blue	0.04
Thymol Blue	0.04
Agar	15.00
Final pH: 8.6 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT, mainly irritating to eyes, respiratory system, and skin. Handle in accordance with good laboratory hygiene and safety practice. Wash hands before breaks and at the end of workday. To protect, use safety glasses and gloves during handling.
3. Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Avoid breathing dust.
4. Do not let product enter drains.
5. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 89.08 g of the medium in one liter of distilled water.
2. Heat with frequent agitation and boil completely to dissolve the medium.
3. DO NOT OVERHEAT. DO NOT AUTOCLAVE.
4. Cool to 45-50°C and pour into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Light beige to green-beige colored, homogeneous, free flowing powder
Solution	8.9% Solution in Distilled or deionized water is soluble on boiling, bluish green / forest green colored, and very slightly to slightly opalescent.
Prepared Medium	Bluish / Forest green, clear and to slightly opalescent gel
Reaction of 8.9% Solution	pH 8.6 ± 0.2 at 25°C
Gel Strength	Firm, compared to 1.5% Agar Gel.

Expected Cultural Response: Cultural response on TCBS Agar, Selective observed after incubation at 35-37°C for 18-48 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Colour of colony
1.	*Enterococcus faecalis* ATCC 29212	>=10^3	Inhibited	0%	-
2.	*Escherichia coli* ATCC 25922	>=10^3	Inhibited	0%	-
3.	*Shigella flexneri* ATCC 12022	>=10^3	Inhibited	0%	-
4.	*Vibrio cholerae* ATCC 15748	50-100	Good luxuriant	>=50 %	Yellow

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5.	*Vibrio parahaemolyticus* ATCC 17802	50-100	Good luxuriant	>=50 %	Blue
6.	*Vibrio fluvialis* ATCC 33809	50-100	Good luxuriant	>=50 %	Yellow
7.	*Vibrio vulnificus* ATCC 29306	50-100	Fair - good	>=30 %	Greenish Yellow

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Observe aseptic techniques.
2. The agar surface should be smooth and moist, but without excessive moisture.
3. Streak the specimen as soon as possible after it is received in the laboratory. Samples may be swabbed directly onto the plated medium.
4. Heavy inoculation is recommended, especially if specimens are not fresh, as the medium is highly selective and vibrios tend to die rather easily.
5. The medium should be inoculated heavily with faecal specimens because growth of few species may be inhibited on the medium due to fermentation of sucrose and accumulation of acids.
6. Swabs containing specimen material should be transported to the laboratory in Cary and Blair Transport Medium[4,6,14] if a delay in reaching the laboratory is anticipated.
7. Specimens for cultivation of vibrios should not be frozen.
8. Incubate plates, protected from light, at 35 ± 2°C in an aerobic atmosphere for 18-24 hours.

Results

1. After 18 – 24 hours of incubation at 32-35°C, sucrose-fermentating vibrios (V. cholerae, V. alginolyticus, V. hareyi, V. cincinnatiensis, V. fluvialis, V. furnissii, V. metschnikovii, and some V. vulnificus) appear smooth, opaque, thin-edged
2. yellow colonies on TCBS Agar.[3]
3. Most other clinically important vibrios, including V. parahaemolyticus, do not ferment sucrose and appear as bluish green colonies.[2]
4. 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]
5. TCBS Agar is highly selective for Vibrio species. However, occasional isolates of Pseudomonas and Aeromonas may also form blue green colonies on TCBS Agar.[9]
6. Any H_2S negative colony of TCBS Agar can be considered presumptive positive for Vibrio.

Typical colonial morphology on TCBS Agar is as follows:

V. cholera ..... Yellow
V. mimicus, V. parahaemolyticus, V. damsel ..... Green
V. vulnificus ..... Green (85%) or yellow (15%)
V. hollisae ..... Green (very poor growth)
V. fluvialis, V. furnissii, V. alginolyticus ..... Yellow
V. metschnikovii ..... Yellow (reduced growth)
Pseudomonas aeruginosa ..... Inhibition, partial to complete; blue
Proteus species ..... Inhibition, partial to complete; yellow to translucent
Escherichia coli ..... Inhibition, partial to complete; translucent

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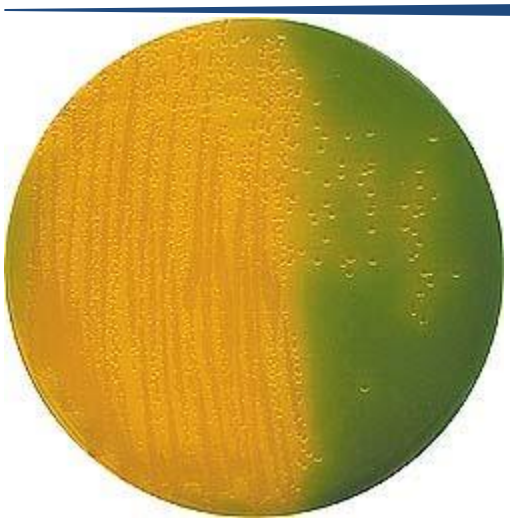

V. cholera ferment sucrose, this results in a pH shift production of yellow colonies.

V. parahaemolyticus do not ferment sucrose and produce bluish green colonies.

Storage
Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

sExpiration
Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure
1. For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification.
2. Colonies that appear yellow on TCBS Agar will produce unsatisfactory oxidase reactions.
3. Sucrose-fermenting *Proteus* spp. produce yellow colonies which may resemble those of *Vibrio*.[8]
4. Consult appropriate texts for detailed information and recommended procedures.[10,11,13-16]

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5. 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.

Packaging
Product Name : TCBS Agar
Product Code : DM376BS
Available Pack sizes : 100gm / 500gm

References

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Further Information

For further information please contact your local MICROMASTER Representative.

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