PRODUCT SPECIFICATION SHEET

Cystine Tryptone Agar (DM068)

Intended Use
Cystine Tryptone Agar (DM068) is recommended for maintenance, subculturing, detection of motility and carbohydrate fermentation studies.

Product Summary and Explanation
Cystine Tryptone Agar is suitable for the propagation and maintenance of bacteria even the fastidious ones without addition of additives. This formulation was developed by Vera as a simple semisolid medium for the identification and maintenance of the *Gonococcus* and other bacteria.[1] Without carbohydrates, it can be used for maintenance of cultures, including fastidious organisms, by a deep stab, like *Brucella, Corynebacterium, Pasteurella, Pneumococci* and *Streptococci* without added enrichments[2-4] for longer periods when stored at appropriate temperatures. Even some light-sensitive anaerobic microorganisms can grow in this medium without special conditions, though in reduced atmospheres, they give ideal growth. This medium has its maximum efficiency when freshly prepared, but if dehydration of the medium is avoided, it can be stored for long period of time. To achieve this, screw caps or proper sealing are strongly recommended. In presence of CO_2, anaerobic organisms like *Actinomyces bovis, Bacteroides funduliformis* and *Leptotrichia* [5] grow well in this medium. With the appropriate carbohydrate, it is recommended for the differentiation of fastidious organisms that do not grow on phenol red classical media, by means of fermentation reactions. In semisolid agar, acid reactions are easily detected because the acid formed is not immediately diffused throughout the entire culture as in broth. Most cultures show an alkaline reaction when no fermentable carbohydrate is present. Motility can be readily detected in the semisolid medium.[6]

Principles of the Procedure
Cystine Tryptone Agar contains casein enzymic hydrolysate, L-cystine which supplies the nutrients necessary to support the growth of fastidious microorganism. Carbohydrate fermentation is detected by a visible colour change of the medium due to the incorporation of the pH indicator dye, phenol red. The medium becomes acidified due to the production of organic acids, when the organism metabolizes the carbohydrate present. However, the peptones present in the medium are also degraded by the bacteria present and yield substances that are alkaline in pH. The phenol red indicator changes from reddish-orange to yellow when the amount of acid produced by carbohydrate fermentation is greater than the alkaline end products of the peptone degradation. The colour change with phenol red occurs around pH 6.8, near the original pH of the medium.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	20.00
L-Cystine	0.50
Sodium chloride	5.00
Sodium sulphite	0.50
Phenol red	0.017
Agar	2.50
Final pH: 7.3 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

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Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 28.51 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Dispense in tubes in 8-10 ml amounts.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Cool to 50°C and add appropriate carbohydrate (0.5 to 1.0% if desired).
6. Mix well and allow the tubed medium to cool in an upright position.

Quality Control Specifications

Dehydrated Appearance	Light yellow to light pink homogeneous free flowing powder
Prepared Medium	Red coloured, clear to slightly opalescent gel forms in tubes as butts
Reaction of 2.85% Solution	pH : 7.3 ± 0.2 at 25°C
Gel Strength	Semisolid, comparable with 0.25% Agar gel.

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 4-18 hours or longer if necessary.

Sr. No.	Organisms		Results to be achieved		
		Inoculum (CFU)	Growth	Motility	Acid in presence of Dextrose
1.	*Escherichia coli ATCC 25922*	50 - 100	good-luxuriant	positive, growth away from stabline causing turbidity	positive reaction, yellow colour
2.	*Neisseria gonorrhoeae ATCC 19424*	50 - 100	Good	negative, growth along the stabline, surrounding medium	positive reaction, yellow colour
3.	*Neisseria meningitides ATCC 13090*	50 - 100	Good	negative, growth along the stabline, surrounding medium	positive reaction, yellow colour
4.	*Streptococcus pneumonia ATCC 6303*	50 - 100	Good	negative, growth along the stabline, surrounding medium	positive reaction, yellow colour

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

Test Procedure

1. Only the surface of the tubed medium is inoculated in case of fermentation studies of the genus *Neisseria*.
2. For facultative organisms, such as *Streptococci* and strictly anaerobic organisms inoculation is done by stabbing the center of the medium with an inoculating needle to about half the depth of the medium.
3. Incubate with loosened caps aerobically or anaerobically depending upon the organisms being tested.

4. *Neisseria* should be incubated with loose caps; if incubated in CO_2 incubator or with tight caps in non-CO_2 incubator.
5. For more rapid growth and also for more rapid fermentation reactions, anaerobic cultures preferably should be incubated in the presence of CO_2 as well as hydrogen or nitrogen. Some strict anaerobes fail to grow or grow poorly in the absence of CO_2.

Results

1. A yellow colour either in the upper one-third or throughout the medium indicates acid production due to carbohydrate fermentation.
2. A red (alkaline) to orange (neutral) colour indicates that the carbohydrate has not been degraded and that only the peptone has been utilized.
3. Inoculated medium (without carbohydrate) also exhibits a red to orange colour.
4. Motile cultures show growth away from the line of inoculation.
5. Non-motile organisms grow in the inoculated area, along the stab line while the surrounding area remains clear.

Storage

Store the sealed bottle containing the dehydrated medium at10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

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. Cystine Tryptone Agar requires a heavy inoculum.
2. Prolonged incubation may lead to changes in pH indicator or abnormal lactose/sucrose reactions with *Neisseria* pathogens.
3. *Neisseria* species usually produce acid only in the area of stabs (upper third). If there is a strong acid (yellow color) throughout the medium, a contaminating organism may be present.
4. Gram stain and oxidase test should be performed on the growth to confirm the presence of *Neisseria* species.
5. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Cystine Tryptone Agar
Product Code : DM068
Available Pack sizes : 100gm/ 500gm

References

1. Vera H. D., 1948, J. Bacteriol. 55:531.
2. Peterson and Hartsell, 1955, J. Inf. Dis., 96:75.
3. Myers and Koshy, 1962, Am. J. Public Health, 96:75.
4. Alford, Wiese and Gunter, 1955, J. Bacteriol. 69:518.
5. Kroeger and Sibal, 1961, J. Bacteriol. 50:581.
6. Vera and Petran, 1954, Bull. Nat. Assoc. Clin. Labs., 5:90

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Further Information

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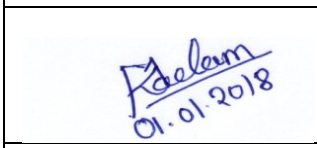	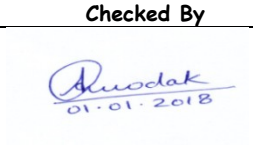	
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