CTA Medium™ • Cystine Tryptic Agar
CTA Medium™ with Carbohydrates

Intended Use

Cystine Tryptic Agar and **CTA Medium** (Cystine **Trypticase**™ Agar Medium) are for the maintenance of microorganisms, as well as for the detection of bacterial motility and, with added carbohydrate, for fermentation reactions of fastidious microorganisms; i.e., *Neisseria*, pneumococci, streptococci and nonsporeforming anaerobes.

Summary and Explanation

This formulation was developed by Vera as a simple semi-solid 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, for extended periods when stored at appropriate temperatures.

With the appropriate carbohydrate, it is recommended for the differentiation of fastidious organisms by means of fermentation reactions. In the semisolid agar, acid reactions are easily detected because the acid formed is not immediately diffused throughout the entire culture as in a broth. When no fermentable carbohydrate is present, most cultures show an alkaline shift.

Motility can be readily detected in the semisolid medium.[2] Stab cultures show growth out from the line of inoculation. Nonmotile organisms grow in the inoculated area, while the surrounding area remains clear.

BBL™ **Taxo**™ carbohydrate discs can be selected and added, as needed, to tubes of plain **CTA Medium** when fermentation reactions are to be determined.

For clostridia, bacilli, common micrococci, enteric bacilli and other organisms not generally considered to be nutritionally fastidious, the use of **Trypticase** Agar Base is recommended instead of this formulation.

Principles of the Procedure

The medium contains cystine and peptone to supply the nutrients necessary to support the growth of fastidious microorganisms.

Carbohydrate fermentation is detected by a visible color change of the medium due to the incorporation of the pH indicator dye, phenol red. When the carbohydrate present is metabolized by the organism, organic acids are produced and the medium becomes acidified. However, the peptone present in the medium is also degraded by the bacteria present and yields substances that are alkaline in pH.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Cystine Tryptic Agar

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	2.85% solution, soluble in purified water upon boiling. Solution is red, very slightly opalescent.
Prepared Appearance:	Red, very slightly opalescent.
Reaction of 2.85% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Cystine Tryptic Agar

Prepare the medium per label directions without and with 0.5% dextrose. Inoculate tubes with fresh broth cultures (*Neisseria* from chocolate agar) by straight stab and incubate with caps tightened at 35 ± 2°C for 18-48 hours (up to 72 hours if necessary).

ORGANISM	ATCC™	MOTILITY	ACID PRODUCTION WITH DEXTROSE
Corynebacterium diphtheriae biotype mitis	8024	–	+
Escherichia coli	25922	+	+
Neisseria gonorrhoeae	43070	–	+

Identity Specifications

BBL™ CTA Medium™

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.85% solution, soluble in purified water upon boiling. Solution is light to medium, red-orange to orange-red to red-rose, clear to slightly hazy.
Prepared Appearance:	Red-orange to red-rose, slightly hazy.
Reaction of 2.85% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ CTA Medium™

Prepare the medium per label directions (without added carbohydrate). Inoculate tubes with fresh broth cultures (*Neisseria* from chocolate agar) by straight stab and incubate at 35 ± 2°C under appropriate atmospheric conditions (*Neisseria* with tightened caps) for 72 hours.

ORGANISM	ATCC™	RECOVERY	MOTILITY
Corynebacterium pseudodiphtheriticum	10700	Good	–
Enterococcus faecalis	29212	Good	–
Listeria monocytogenes	19115	Good	+
Neisseria gonorrhoeae	19424	Good	–
Neisseria meningitidis	13090	Good	–
Staphylococcus aureus	6538P	Good	–

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 peptone degradation. The color change with phenol red occurs around pH 6.8, near the original pH of the medium.

Formulae

Difco™ Cystine Tryptic Agar

Approximate Formula* Per Liter

Tryptose	20.0	g
L-Cystine	0.5	g
Sodium Chloride	5.0	g
Sodium Sulfite	0.5	g
Agar	2.5	g
Phenol Red	17.0	mg

BBL™ CTA Medium™

Approximate Formula* Per Liter

Pancreatic Digest of Casein	20.0	g
L-Cystine	0.5	g
Sodium Chloride	5.0	g
Sodium Sulfite	0.5	g
Agar	2.5	g
Phenol Red	17.0	mg

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

Directions for Preparation from Dehydrated Product

Difco™ Cystine Tryptic Agar

1. Suspend 28.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at not over 118°C for 15 minutes.
4. To prepare fermentation medium, add 5-10 g of carbohydrate before autoclaving or dissolve medium in 900 mL water, autoclave, and aseptically add 100 mL sterile 5-10% carbohydrate solution.
5. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ CTA Medium™

1. Suspend 28.5 g of the powder in 1 L of purified water. Add carbohydrate (0.5 to 1.0%) if desired, and adjust the pH if necessary. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute or until solution is complete.
3. Tube and autoclave at not over 118°C for 15 minutes. Cool in the upright position.
4. Store at room temperature. Do not refrigerate unless in tightly closed, screw-capped tubes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Loosen caps, boil, tighten caps and cool before use.
2. Remove fresh colony growth from the surface of a suitable culture medium; e.g., Chocolate Agar, not from a selective, primary isolation plate.[3]
3. For fermentation tests with members of the genus *Neisseria*, only the surface of the tubed medium is inoculated. For facultative organisms, such as streptococci and strictly anaerobic organisms, inoculate by stabbing the center of the medium with an inoculating needle to about 1/2 the depth of the medium.
4. Repeat for each tube to be inoculated.
5. Incubate at 35 ± 2°C with loosened caps aerobically or anaerobically depending upon the organisms being tested; *Neisseria* should be incubated with tight caps[4] especially if tubes must be incubated in a CO_2 incubator,[5,6] or with loose caps in a non-CO_2 incubator.[7,8] Examine periodically up to 24 hours for growth (turbidity), evidence of motility, and acid production in carbohydrate-containing medium (yellow color in upper layer of medium). A few strains may require incubation for up to 48-72 hours.[9]
6. Many fastidious organisms, including *Neisseria*, *Pasteurella*, streptococci, *Brucella*, corynebacteria and vibrios, may be readily cultivated in this medium, no added carbon dioxide, serum or other enrichments being required.
7. For more rapid growth and also for more rapid fermentation reactions, anaerobic cultures preferably should be incubated in the presence of carbon dioxide as well as hydrogen or nitrogen. Some strict anaerobes fail to grow or grow poorly in the absence of carbon dioxide.

Expected Results

A yellow color either in the upper one-third or throughout the medium indicates acid production; i.e., fermentation of the carbohydrate. A red (alkaline) to orange (neutral) color indicates that the carbohydrate has not been degraded and that only the peptone has been utilized. Inoculated medium (without carbohydrate) also exhibits a red to orange color.

Motile organisms show growth out from the line of stab-inoculation. Nonmotile organisms only grow along the stab line with the surrounding agar remaining clear.

Limitations of the Procedure

1. CTA requires a heavy inoculum.[10]
2. Prolonged incubation may lead to changes in pH indicator or abnormal lactose/sucrose reactions with *Neisseria* pathogens.[11,12]
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. If in doubt about a tube containing a *Neisseria* species, a Gram stain and oxidase test should be performed on the growth.[10]

References

1. Vera. 1948. J. Bacteriol. *55*:531.
2. Vera and Petran. 1954. Bull. Nat. Assoc. Clin. Labs. *5*:90.
3. Morello, Janda and Doern. 1991. *In* Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
4. Kellogg. 1974. *In* Lennette, Spaulding and Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
5. Yu and Washington. 1985. *In* Washington (ed.), Laboratory procedures in clinical microbiology, 2nd ed. Springer-Verlag, New York, N.Y.
6. Morse and Knapp. 1987. *In* Wentworth (ed.), Diagnostic procedures for bacterial infections, 7th ed. American Public Health Association, Washington, D.C.

7. Center for Disease Control. 1978. Laboratory methods in clinical bacteriology. CDC, Atlanta, Ga.
8. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, Mo.
9. Finegold and Martin. 1982. Bailey & Scott's diagnostic microbiology, 6th ed. The C.V. Mosby Company, St. Louis, Mo.
10. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
11. Faur, Weisburd and Wilson. 1975. J. Clin. Microbiol. *1*:294.
12. Applebaum and Lawrence. 1979. J. Clin. Microbiol. *9*:598.

Availability

Difco™ Cystine Tryptic Agar

AOAC

Cat. No.	252310	Dehydrated – 500 g

BBL™ CTA Medium™

AOAC

Cat. No.	211096	Dehydrated – 500 g
	221631	Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10*
	221632	Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100*

BBL™ CTA Medium™ with Carbohydrates

Cat. No.	297731	Prepared Tubes with Arabinose – Pkg. of 10*
	297732	Prepared Tubes with Cellobiose – Pkg. of 10*
	221633	Prepared Tubes with Dextrose – Pkg. of 10*
	221634	Prepared Tubes with Dextrose – Ctn. of 100*
	296001	Prepared Tubes with Fructose – Pkg. of 10*
	221635	Prepared Tubes with Lactose – Pkg. of 10*
	221637	Prepared Tubes with Maltose – Pkg. of 10*
	221639	Prepared Tubes with Mannitol – Pkg. of 10*
	297101	Prepared Tubes with Raffinose – Pkg. of 10*
	297102	Prepared Tubes with Rhamnose – Pkg. of 10*
	221641	Prepared Tubes with Salicin – Pkg. of 10*
	221643	Prepared Tubes with Sorbitol – Pkg. of 10*
	296002	Prepared Tubes with Starch – Pkg. of 10*
	221645	Prepared Tubes with Sucrose – Pkg. of 10*
	297033	Prepared Tubes with Trehalose – Pkg. of 10*
	221647	Prepared Tubes with Xylose – Pkg. of 10*

**Store at 2-8°C.*