

BBL[™] CTA Medium[™] BBL[™] CTA Medium[™] with Dextrose BBL[™] CTA Medium[™] with Lactose BBL[™] CTA Medium[™] with Maltose L007450 • Rev. 10 • April 2015

QUALITY CONTROL PROCEDURES

I INTRODUCTION

CTA Medium[™] (Cystine **Trypticase**[™] Agar Medium) is a simple, basic medium for the maintenance and detection of motility of a wide variety of microorganisms. With added carbohydrates, it is useful for the determination of fermentation reactions.

II PERFORMANCE TEST PROCEDURE

- 1. Inoculate representative samples with the cultures listed below.
 - a. Loosen caps, boil the media in a boiling water bath* for approximately 2 min, tighten caps and cool before use. ***NOTE:** Use of a microwave oven is not recommended.
 - b. Inoculate the tubes using an inoculating needle to stab into the middle of the column of medium to a depth approximately one-half the total depth of the medium. For the Neisseria cultures, use a 0.01 mL calibrated loop and inoculate the surface of the medium using three or four colonies from a 24- to 48-h Chocolate II Agar plate. For the other test cultures, use 10⁻¹ dilutions of 18- to 24-h Trypticase Soy Broth cultures.
 - c. Incubate all tubes at 35 ± 2 °C. Incubate the *Neisseria* culture with tightened caps. Incubate the remainder with loosened caps in an aerobic atmosphere.
- 2. Examine tubes after 18 24 and 42 48 h for growth and reactions.

3. Expected Results

Cultures of the organisms in the table below are recommended for checking the performance of **CTA Medium** containing the various carbohydrates. The final colors produced should be compared with the color of the respective uninoculated medium.

Organisms	ATCC ®	Base*	Dextrose	Lactose	Maltose
Corynebacterium pseudodiphtheriticum	10700		K		
Listeria monocytogenes	19115	MK			
Neisseria gonorrhoeae	19424		A	K	K
Neisseria meningitidis	13090				А
Staphylococcus aureus	25923			А	
Neisseria lactamica	23970			А	

A = Acid (yellow); K = no reaction (red to reddish orange); M = motile

* The base contains no carbohydrate. It is included to indicate a system failure such as a carry-over of carbohydrate from a primary isolation medium.

III ADDITIONAL QUALITY CONTROL

- 1. Examine tubes as described under "Product Deterioration."
- 2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
- 3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.6 ± 0.2 (with lactose, 7.3 ± 0.2).
- 4. Incubate uninoculated representative tubes at 20 25 °C and 30 35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

CTA Medium is a culture medium for the maintenance of microorganisms. It is also used for the detection of bacterial motility and, with added carbohydrate, for fermentation reactions of fastidious microorganisms, i.e., *Neisseria*, pneumococci, streptococci and nonsporeforming anaerobes.

V SUMMARY AND EXPLANATION

CTA Medium was developed by Vera as a simple medium for the identification and maintenance of the gonococcus and other bacteria.¹ **CTA Medium** without carbohydrates can be used for maintenance of cultures, including fastidious organisms, for extended periods when stored at appropriate temperatures.

CTA Medium with the appropriate carbohydrate 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. 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.

BBLTM **Taxo**TM Carbohydrate Discs can conveniently 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 **CTA Medium**.

VI PRINCIPLES OF THE PROCEDURE

CTA Medium contains cystine and casein 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. The peptone present in the medium, however, is also degraded by the bacteria present and yields substances which 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 peptone degradation.³ The color change with phenol red occurs around pH 6.8.

VII REAGENTS

CTA Medium

Approximate Formula* Per Liter Purified Water

L-Cystine0.5 g	Sodium Chloride5.0 g
Pancreatic Digest of Casein 20.0 g	Sodium Sulfite0.5 g
Agar	Phenol Red0.017 g

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

CTA Medium with Dextrose, Lactose or Maltose contains the above ingredients with, per liter, 5.0 g of the specified carbohydrate. **Warnings and Precautions:** For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2 - 8 °C. Avoid freezing and overheating. Do not open until ready to use. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Minimize exposure to light.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{4,5} Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: CTA Medium and/or CTA Medium with Dextrose, Lactose or Maltose

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

- 1. Loosen caps, boil media in a boiling water bath* for approximately 2 min, 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.⁶
- 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 half 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 organism being tested; *Neisseria* should be incubated with tight caps,^{3,7} especially if tubes must be incubated in a CO₂ incubator,^{8,9} or with loose caps in a non-CO₂ incubator.^{10,11} Examine periodically up to 24 h for growth (turbidity), evidence of motility, and acid production (yellow color in upper layer of medium). A few strains may require incubation for up to 48 72 h.¹²
- 6. Many fastidious organisms, including *Neisseria, Pasteurella*, streptococci, *Brucella*, corynebacteria and vibros may be readily cultivated in **CTA Medium**, no added carbon dioxide, serum, or other enrichments being required.
- 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.

***NOTE:** Use of a microwave oven is not recommended.

User Quality Control: See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X 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 plain **CTA Medium** (without carbohydrates) 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.

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.^{4,5,13}

XII PERFORMANCE CHARACTERISTICS

Pizzuto and Washington compared three procedures used for the identification of *Neisseria*: a modified rapid fermentation test; the **BACTEC™** *Neisseria* differentiation kit; and the **CTA Medium** technique. The 156 clinical isolates tested consisted of 101 strains of *N. gonorrhoeae*, 45 strains of *N. meningitidis*, 4 strains of *N. lactamica*, 2 strains of *N. subflava*, 1 strain of *N. sicca*, 2 strains of *Branhamella catarrhalis* and 1 strain of CDC group II F. Although the CTA method required up to 48 h of incubation, overall it was the most accurate identifying 96% of gonococci and 100% of other *Neisseria* species.¹⁴

XIII AVAILABILITY

Cat. No. Description

221631 BD BBL™ CTA Medium™, 8 mL, Pkg. of 10 size K tubes

- 221632 BD BBL™ CTA Medium™, 8 mL, Ctn. of 100 size K tubes
- 221633 BD BBL™ CTA Medium™ with Dextrose, Pkg. of 10 size K tubes
- 221634 BD BBL™ CTA Medium™ with Dextrose, Ctn. of 100 size K tubes
- 221635 BD BBL™ CTA Medium™ with Lactose, Pkg. of 10 size K tubes
- 221637 BD BBL™ CTA Medium™ with Maltose, Pkg. of 10 size K tubes

XIV REFERENCES

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Technical Information: In the United States, contact BD Technical Service and Support at 800-638-8663 or www.bd.com/ds.

Becton, Dickinson and Company 7 Loveton Circle Sparks, MD 21152 USA

EC REP

Benex Limited Pottery Road, Dun Laoghaire Co. Dublin, Ireland

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