

Product Insert

CYCLOSERINE CEFOXITIN MANNITOL BROTH WITH TAUROCHOLATE AND LYSOZYME (CCMB-TAL)

Products

AS-8216 Cycloserine Cefoxitin Mannitol Broth with Taurocholate and Lysozyme (CCMB-TAL) 10 tubes / pkg

Intended Use

Cycloserine Cefoxitin Mannitol Broth with Taurocholate and Lysozyme (CCMB-TAL) is an enriched, selective, and differential broth media used for the isolation and presumptive identification of *Clostridium difficile*, a recognized cause of pseudomembranous (antimicrobial agent-associated) colitis.

Summary

CCMB-TAL is an enriched, selective, and differential broth media used for the isolation of *Clostridium difficile*. The nutritive base consists of animal peptones and mannitol. This media is supplemented with cefoxitin and cycloserine at concentrations that inhibit the growth of most normal fecal flora. Cycloserine will inhibit gram-negative bacteria, while cefoxitin will inhibit both gram-positive and gram-negative organisms. *Clostridium difficile* is not inhibited in the CCMB-TAL media, and when grown, will exhibit a characteristic smell. Neutral red is added as a pH indicator. The presence of *C. difficile* will turn the indicator from red/orange to yellow due to the organism utilizing the amino acids and causing an increase in the pH. Sodium Taurocholate and lysozyme are added to help in the germination of spores. Cysteine is added as a reducing agent. This media is prepared, dispensed, and packaged under oxygen-free conditions to prevent the formation of oxidized products prior to use.

Formulation*

Proteose Peptone	40.00	g
Sodium Phosphate Dibasic	5.00	g
Potassium Phosphate Monobasic	1.00	g
Sodium Chloride	2.00	g
Magnesium Sulfate Heptahydrate	0.20	g
Mannitol	6.00	g
Sodium Taurocholate	1.00	g
Neutral Red (0.3% solution)	4.00	mL
L-Cysteine Hydrochloride (25.0% solution)	2.00	mL
Cycloserine (10.0% solution)	5.00	mL
Cefoxitin (1.56% solution)	1.00	mL
Lysozyme (1.0% solution)	0.50	mL
DI Water	1.00	L

Final pH: 7.3 ± 0.3 at 25°C

Final volume: 5.0 mL ± 0.5 mL

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

Precautions

For *IN VITRO DIAGNOSTIC USE* only. Utilize approved biohazard precautions and aseptic technique when using this product. This product is for use only by properly trained and qualified personnel. Sterilize all biohazard waste prior to disposal.

Storage and Shelf Life

Storage: Upon receipt, store at 2 – 8°C in original container until used. Avoid overheating or freezing. Do not use media if there are signs of deterioration (shrinking, cracking, or discoloration due to oxidation of media) or contamination. The expiration date applies to the product in its original packaging and stored as directed. Do not use product past the expiration date shown on the container.

Shelf Life: 90 days from date of manufacture at 2 – 8°C.

Procedure

Specimen Collection: Protect specimens for anaerobic culture from oxygen during collection, transportation, and processing. Consult appropriate references for detailed instructions concerning collection and transport of anaerobes.

Methods for Use: Remove CCMB-TAL from 2 - 8°C environment and allow to come up to room temperature. CCMB-TAL should be inoculated directly with clinical specimens or from a broth that has been previously inoculated from a clinical specimen. Inoculate tubes and immediately place in an anaerobic atmosphere, incubating at 35-37°C for 18-48 hours. Subculture tubes that have turned a yellow color, which is indicative of a pH change, for isolation of *C. difficile*. Recovery of *C. difficile* from an inoculated tube can be achieved by plating 10-100µL of the specimen in an anaerobic environment onto a selective media such as CCFA (Catalog #: AS-213) or a non-selective media such as BRU (catalog #: AS-111). Detailed instructions for processing anaerobic cultures can be found in the appropriate references.

Materials Required, But Not Provided

Standard microbiological supplies and equipment such as loops, saline blanks, slides, staining supplies, microscope, incinerator / autoclave, incubators, anaerobic chamber / anaerobic jars, other culture media, and serological / biochemical reagents.

Interpretation of Results

If used properly, CCMB-TAL supports good growth of species of *C. difficile*. After 24-48 hours, the tube should be yellow in color from the increase in pH due to the utilization of amino acids by *C. difficile*. When recovered on plated media, these colonies fluoresce golden yellow/chartreuse under long-wavelength UV light. Most other bacteria are inhibited in this media. After 48 hours, other organisms such as *Lactobacilli*, *Clostridia*, and yeast, may grow and can increase the pH changing the indicator to yellow.

Limitations

CCMB-TAL will not provide complete information for identification of bacterial isolates. Rare strains of *C. difficile* may be inhibited. Tubes should be examined after 24 and 48 hours of incubation to insure optimal selectivity. After 48 hours of incubation, significant numbers of isolates other than *C. difficile* may grow. A test for aerotolerance should be used to confirm that each colony type is an obligate anaerobe. Consult reference materials for additional information.

Quality Control

The following organisms are routinely used for quality control performance testing at Anaerobe Systems.

Organism Tested	ATCC #	Results	Time	Special Reaction
Bacteroides fragilis	25285	No Growth		
Enterococcus faecalis	29212	No Growth		
Clostridium sporogenes	3584	No Growth		
Clostridium beijerinckii	8260	No Growth		
Proteus mirabilis	12453	No Growth		
Clostridium perfringens	13124	No Growth		
Clostridium innocuum	14501	No Growth		
Clostridium sordellii	9714	No Growth		
Clostridium difficile	9689	Growth	24 hrs	Yellow coloration
Clostridium difficile	700057	Growth	24 hrs	Yellow coloration

User Quality Control: The final determination to the extent and quantity of user laboratory quality control must be determined by the end user.

If sterility testing is to be performed on this product, a representative sample of the lot(s) should be incubated anaerobically and aerobically for 48 – 96 hours.

If the nutritive/inhibitory capacity of this media is to be tested for performance, it is recommended that the following ATCC organisms be evaluated for growth/inhibition.

Organism	ATCC #	Expected Growth	Special Reaction
B. fragilis	25285	Inhibited	
E. faecalis	29212	Inhibited	
S. aureus	25923	Inhibited	
C. difficile	9689	24 hours	Yellow coloration

Physical Appearance: CCMB-TAL should appear as a clear red liquid.

References

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