

Procedure

1. Sample collection, storage and subculturing to plated medium.⁷

Place rectal swab about 1 cm into the medium and twirl the swab. Remove the swab or lower it to bottom of the tube and break the shaft of the swab with the lip of the tube to allow easy access to the shaft.

With solid stools, prepare a saline suspension, blend in a mechanical mixer (i.e., vortex) and place five drops into the medium about 1 cm below the surface. Alternatively, probe all areas of the stool with a swab and inoculate the medium as described for a rectal swab. With diarrheal stools, place five drops in the medium about 1 cm below the surface.

Refrigerate inoculated Campylobacter Thioglycollate Medium overnight and subculture the next day to Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood plates using a Pasteur pipette inserted about 2 cm below the surface of the broth to continuously withdraw a sample as the tip is slowly drawn to the surface. Do not subculture onto nonselective media since the normal flora may still be viable.

2. Incubation of plated medium.

Incubate plated medium at 42°C in a reduced oxygen, increased carbon dioxide atmosphere. This atmosphere can be achieved by using one BBL™ CampyPak™ or CampyPak™ Plus disposable gas generator envelope in a GasPak™ 100 jar, three envelopes in a GasPak™ 150 jar or using the BBL™ CampyPouch™, Bio-Bag™ Type Cfj or GasPak EZ Campy systems. Alternatively, the atmosphere can be achieved using evacuation of GasPak vented jars and replacement with cylinder gases,⁸ or by using the Fortner principle.⁹

Expected Results

Plates of Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood inoculated from Campylobacter Thioglycollate Medium with 5 Antimicrobics should be examined for the presence of colonies of *Campylobacter jejuni*. These colonies will appear as small, mucoid, usually grayish in coloration, flat with irregular edges and nonhemolytic at 24 and 48 hours.¹⁰

Colonies may be only barely visible at 18 and 24 hours. An alternate colonial morphology, which appears to be strain related, consists of round colonies 1-2 mm in diameter, which are convex, entire and glistening.¹⁰ A small percentage of strains may appear tan or slightly pinkish in coloration.⁷

Colonies tend to spread or swarm, especially when initially isolated from fresh clinical specimens.

NOTE: If plates are examined after 24 hours of incubation, treat plates as if they were anaerobic cultures; i.e., examine plates quickly and place them back into a reduced oxygen atmosphere immediately after examination.

References

1. Dekeyser, Gossuin-Detrain, Butzler and Sternon. 1972. *J. Infect. Dis.* 125:390
2. Skirrow. 1977. *Br. Med. J.* 2:9.
3. Blaser, Cravens, Powers and Wang. 1978. *Lancet* 2:979
4. Blaser, Berkowitz, LaForce, Cravens, Reller and Wang. 1979. *Ann. Intern. Med.* 91:179.
5. Luechtefeld, Wang, Blaser and Reller. 1981. *J. Clin. Microbiol.* 13:438
6. Reller, Wang and Blaser. 1979. *Campylobacter enteritis: Campylobacter fetus subspecies jejuni*. ASCP Check Sample, Microbiology No. MB-99, Commission on Continuing Education, American Society of Clinical Pathologists, Chicago, Ill.
7. Kaplan. 1980. *In*. Lennette, Balows, Hausler and Truant (ed.), *Manual of Clinical Microbiology*, 3rd ed. American Society for Microbiology, Washington, D.C.
8. Nachamkin, 1999. *In* Murray, Baron, Pfaller, Tenover and Tenover (ed.), *Manual of clinical microbiology*, 7th ed. American Society for Microbiology, Washington, D.C.
9. Karmali and Fleming. 1979. *J. Clin. Microbiol.* 10:245.
10. Smibert. 1984. *In* Kreig and Holt (ed.), *Bergey's Manual™ of systematic bacteriology*, vol. 1, Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Campylobacter Thioglycollate Medium with 5 Antimicrobics

Cat. No.	221747	Prepared Tubes – Pkg. of 10
	221748	Prepared Tubes – Ctn. of 100

Candida BCG Agar Base Candida Bromcresol Green Agar

Intended Use

Candida Bromcresol Green (BCG) Agar is a differential and selective medium used for primary isolation and detection of *Candida* species from clinical specimens.

Summary and Explanation

Candida BCG medium employs the formula devised by Harold and Snyder.¹ They demonstrated that the triphenyltetrazolium chloride (TTC) being used as an indicator in Pagano Levin medium retarded the growth of some species of *Candida* and completely inhibited the growth of others. To overcome this, they replaced TTC with bromcresol green, a non-toxic indicator, to develop Candida BCG Agar. Neomycin is incorporated to inhibit gram-negative and some gram-positive bacteria.

Principles of the Procedure

This medium consists of peptone agar base supplemented with yeast extract and dextrose to provide the nutrients necessary to support growth. Neomycin is an aminoglycoside antibiotic that is active against aerobic and facultatively anaerobic gram-negative bacteria and certain gram-positive species. Bromcresol green aids in differentiation and identification of *Candida* species based on dextrose fermentation. A change in the pH causes the medium to become a yellow color around the colonies of organisms that ferment dextrose.

User Quality Control

Identity Specifications

Difco™ Candida BCG Agar Base

Dehydrated Appearance:	Beige to blue-green, free-flowing, homogeneous.
Solution:	6.6% solution, soluble in purified water upon boiling. Solution is blue-green to green-blue, slightly opalescent to opalescent, may have a precipitate.
Prepared Appearance:	Blue-green to green-blue, slightly opalescent to opalescent.
Reaction of 6.6% Solution at 25°C:	pH 6.1 ± 0.1

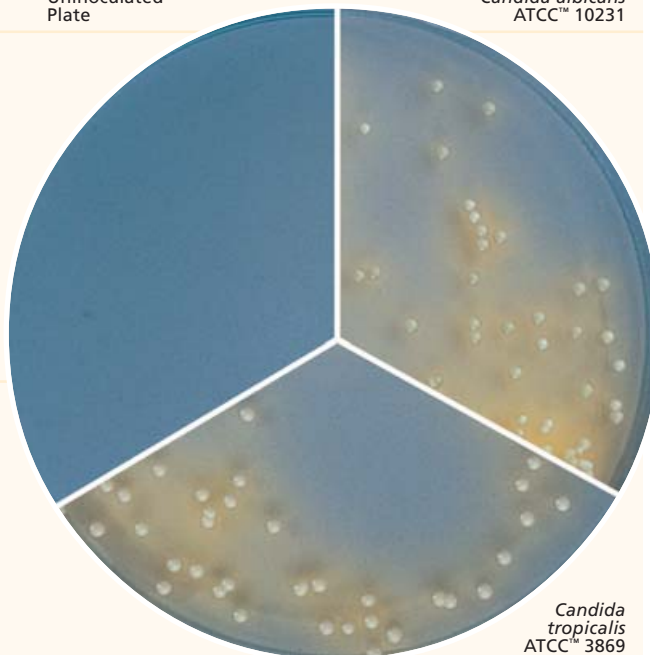
Cultural Response

Difco™ Candida BCG Agar Base

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 24-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLOR OF MEDIUM
<i>Candida albicans</i>	10231	10 ² -10 ³	Good	Yellow
<i>Candida tropicalis</i>	9968	10 ² -10 ³	Good	Yellow
<i>Escherichia coli</i>	25922	10 ³	Inhibition	Green

Uninoculated Plate

Candida albicans
ATCC™ 10231

Formula

Difco™ Candida BCG Agar Base

Approximate Formula* Per Liter

Peptone	10.0	g
Yeast Extract	1.0	g
Dextrose	40.0	g
Agar	15.0	g
Bromcresol Green	0.02	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 66 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 121°C for 15 minutes.
4. Add sterile neomycin (500 µg/mL) to the medium at 50-55°C. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate the plates in an inverted position (agar side up) at 30 ± 2°C for up to 72 hours.

Expected Results

Candida species produce convex to cone-shaped, smooth to rough colonies. The color of the medium around the colonies becomes yellow, usually within 72 hours.

Gram staining, biochemical tests and serological procedures should be performed to confirm findings.²⁻⁴

References

1. Harold and Snyder. 1968. Personal communication.
2. Kwon-Chung and Bennett. 1992. Medical mycology. Lea & Febiger, Philadelphia, Pa.
3. Forbes, Sahn and Weissfeld. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis, Mo.
4. Warren and Hazen. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Candida BCG Agar Base

Cat. No. 283510 Dehydrated – 500 g

BBL™ Candida Bromcresol Green Agar

Cat. No. 296241 Prepared Plates (complete) – Pkg. of 20*

*Store at 2-8°C.