EC Medium, Modified Novobiocin Antimicrobic Supplement

Intended Use

EC Medium, Modified is used with Novobiocin Antimicrobic Supplement in the detection of *Escherichia coli* O157:H7 in meat and poultry products.

Summary and Explanation

EC Medium, Modified and Novobiocin Antimicrobic Supplement are based on the formula for modified EC broth with novobiocin (mEC+n) as described by Okrend and Rose.¹ In modifying the EC Medium formula, Okrend and Rose reduced the Bile Salts No. 3 from 1.5 g per liter to 1.12 g per liter and added 20 mg per liter of sodium novobiocin. Okrend et al. reported that mEC+n was useful in the enrichment and detection of *E. coli* O157:H7 from meats and poultry products.²⁻⁴

Principles of the Procedure

Peptone supports good growth of *E. coli* O157:H7 and is rich in peptides and nitrogen. Lactose is an additional source of carbon for organisms, such as *E. coli*, that can ferment this sugar. Dipotassium phosphate and monopotassium phosphate are buffers that facilitate recovery of injured cells. Sodium chloride provides a suitable ionic environment for growth of microorganisms.

Selectivity of the medium is achieved by the incorporation of Bile Salts No. 3 into the base medium and by the addition of sodium novobiocin to the complete medium. These agents suppress the growth of nuisance organisms commonly found in foods. The sodium novobiocin is provided in the freeze-dried state as Novobiocin Antimicrobic Supplement. This supplement is rehydrated before use with sterile purified water.

User Quality Control

Identity Specifications Difco[™] EC Medium, Modified

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.		
Solution:	3.66% solution, soluble in purified water. Solution is light to medium amber, clear.		
Prepared Appearance:	Light to medium amber, clear.		
Reaction of 3.66% Solution at 25°C:	рН 6.9 ± 0.2		
Difco™ Novobiocin Antimicrobic Supplement			
Lyophilized Appearance:	White cake.		
Rehydrated Appearance:	Colorless solution.		

Cultural Response Difco™ EC Medium, Modified

Prepare the medium (without added novobiocin) per label directions. Inoculate and incubate at $35 \pm 2^{\circ}$ C for a maximum of 24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecalis	33186	10 ³	None to poor
Escherichia coli O157:H7	35150	10-10 ²	Good

Formulae

Difco[™] EC Medium, Modified

Approximate Formula* Per Liter Pancreatic Digest of Casein	0.0	a
Bile Salts No. 3	1.12	g
Lactose	5.0	g
Dipotassium Phosphate	4.0	g
Monopotassium Phosphate	1.5	g
Sodium Chloride	5.0	g
Difco™ Novobiocin Antimicrobic Supplement		

Formula Per 10 mL Vial		
Sodium Novobiocin	20.0	mg
*Adjusted and/or supplemented as required to meet performance criteria.		

Directions for Preparation from Dehydrated Product Difco[™] EC Medium, Modified

- 1. Dissolve 36.6 g of the powder in 1 L of purified water.
- 2. Autoclave at 121°C for 15 minutes. Cool to room temperature.
- 3. Aseptically add 10 mL rehydrated Novobiocin Antimicrobic Supplement. Mix well.
- 4. Test samples of the finished product for performance using stable, typical control cultures.

Difco[™] Novobiocin Antimicrobic Supplement

- 1. Aseptically add 10 mL sterile purified water to the vial.
- 2. Shake to dissolve the contents.

Procedure

Many procedures and systems have been described for the use of mEC+n in the selective and differential enrichment of *E. coli* O157:H7 in meat and poultry samples. Consult appropriate references.⁴⁶ The procedure for the enrichment and detection of *E. coli* O157:H7 in meat and poultry samples using mEC+n described here was in use by the USDA.²⁴ More recently, the USDA has replaced mEC+n with modified TSB with novobiocin plus casamino acids (mTSB+n).

- 1. Inoculate 25 g of meat sample into 225 mL of EC Medium, Modified, with novobiocin in a stomacher bag. Blend or stomach as required (i.e., 2 minutes) for thorough mixing.
- 2. Incubate at 35°C for 24 hours.
- Dilute cultures 10-fold in Butterfield's Phosphate Diluent and inoculate 0.1 mL of appropriate dilutions using a spread plate technique onto MacConkey Sorbitol Agar (MSA) and MacConkey Sorbitol Agar with BCIG (5-bromo-4-chloro-3-indoxyl-β-D-glucuronide) agar plates.
- 4. Incubate plates at 42°C for 24 hours.
- 5. Examine MSA plates for sorbitol-negative colonies (white) and MSA-BCIG plates for sorbitol-negative, BCIG-negative colonies (white).
- Subculture sorbitol-negative colonies to respective plates of EMB Agar and Phenol Red Sorbitol (PRS) Agar containing MUG (4-methylumbelliferyl-β-D-glucuronide).



7. Incubate EMB and PRS-MUG Agar plates at 35°C for 18-24 hours. Examine plates for sorbitol fermentation, MUG reaction (fluorescence), and typical E. coli growth on EMB Agar.

Expected Results

Growth in EC Medium, Modified, with novobiocin is demonstrated as an increase in turbidity. Colonies of E. coli O157:H7 appear white on MacConkey Sorbitol and MacConkey Sorbitol-BCIG Agars. Fermentation of sorbitol in Phenol Red Sorbitol Broth is demonstrated by the production of a yellow color in the medium. With sorbitol nonfermenters, the color of the medium remains red to reddish purple. Positive MUG reactions are demonstrated as a blue fluorescence in the medium under long-wave UV light. Colonies of E. coli on EMB Agar appear blue-black to dark purple. A green metallic sheen may also be present.

Cultures that are sorbitol-negative, MUG-negative and produce blue-black to dark purple colonies with a green metallic sheen on EMB Agar are indicative of E. coli O157:H7. These cultures should be tested serologically and with additional biochemical testing to confirm their identity as E. coli O157:H7.

References

- Okrend and Rose. 1989. Isolation and identification of E. coli O157:H7 from meat. USDA Food Safety Inspection Service. Rev. 3 of Laboratory Communication no. 38. E. coli O157:H7. 20 December 1989. U.S. Department of Agriculture, Washington, D.C.
- 2. Okrend, Rose and Bennett. 1990. J. Food Prot. 53:249.
- Okrend, Rose and Lattuada. 1990. J. Food Prot. 53:941.
 Okrend, Rose and Matner. 1990. J. Food Prot. 53:936.
- Hawkins and Orme. 1995. Proc. Vest. Sec., Amer. Soc. Animal Sci. vol. 46.
 Johnson, Durham, Johnson and MacDonald. 1995. Appl. Environ. Microbiol. 61:386.

Availability

Difco[™] EC Medium, Modified

CCAM COMPF

Cat. No.	234020	Dehydrated – 500 g
	234010	Dehydrated – 2 kg
	234001	Dehydrated – 10 kg

Difco[™] Novobiocin Antimicrobic Supplement

CCAM COMPF

Cat. No. 231971 Vial - 6 × 10 mL* *Store at 2-8°C.

