Directions for Preparation from Dehydrated Product

1. Suspend 23 g of the powder in 1 L of purified water.
2. Heat to boiling. Avoid overheating. DO NOT AUTOCLAVE.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For feces and other solid materials, suspend 1-2 g of the specimen in the broth (approximately 10-15% by volume) and emulsify with an inoculating needle, if necessary.

User Quality Control

Identity Specifications

Difco™ Selenite Broth

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	2.3% solution, soluble in purified water upon boiling. Solution is very light amber, clear to very slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Very light amber, clear to very slightly opalescent, may have a slight precipitate.
Reaction of 2.3% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Selenite Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours. After incubation, subculture onto MacConkey Agar plates and incubate plated media at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONIES ON MACCONKEY AGAR
Escherichia coli	25922	10^2-10^3	Partial inhibition	Pink with bile precipitate
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhimurium	14028	10^2-10^3	Good	Colorless

Incubate tubes with loosened caps at 35 ± 2°C for up to 24 hours. Subcultures should be made after 12-18 hours of incubation, if possible. Coliforms will tend to overgrow the pathogens if incubated longer than 24 hours.

Expected Results

After incubation, there should be an increase in the number of pathogens that the medium is designed to select for and enrich. Subculture onto appropriate selective and differential media (e.g., MacConkey Agar, Hektoen Enteric Agar, XLD Agar, XLT4 Agar, **CHROMagar**™ Salmonella) to isolate pathogens for identification.

Limitation of the Procedure

Enrichment broths should not be used as the sole isolation medium. They are to be used in conjunction with selective and nonselective plating media to increase the probability of isolating pathogens, especially when they may be present in small numbers. Consult references for detailed information and recommended procedures.[3]

References

1. Leifson. 1936. Am. J. Hyg. *24*:423.
2. Taylor and Harris. 1965. Am. J. Clin. Pathol. *44*:476.
3. Nachamkin. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Selenite Broth

BS10 MCM7 SMWW

Cat. No. 227540 Dehydrated – 500 g

BBL™ Selenite-F Broth

BS10 MCM7 SMWW

Cat. No. 221020 Prepared Tubes (K Tubes), 8 mL – Pkg. of 10*
221021 Prepared Tubes (K Tubes), 8 mL – Ctn. of 100*

**Store at 2-8°C.*

Selenite Cystine Broth

Intended Use

This medium conforms with specifications of *The United States Pharmacopeia* (*USP*).

Selenite Cystine Broth is used as a selective enrichment medium for the isolation of *Salmonella* from feces, foods, pharmaceutical articles, water and other materials of sanitary importance.

Summary and Explanation

Leifson found that selenite inhibited fecal streptococci and coliforms during the first 8-12 hours of incubation, thereby permitting salmonellae to replicate without overwhelming interference from other members of the intestinal flora.[1] North and Bartram modified Leifson's Selenite-F Enrichment broth by adding cystine, which stimulated growth of *Salmonella*.[2]

The cystine-containing formulation is included in the *USP* for use in the performance of Microbial Limit Test procedures for *Salmonella* species and is recommended by the Food and Drug Administration, AOAC International and American Public Health Association for detecting *Salmonella* in foods and waters.[3-7]

Selenite Cystine Broth and similar enrichment media are useful for detecting *Salmonella* in the nonacute stages of illness when the organisms occur in the feces in low numbers and for epidemiological studies to enhance the detection of low numbers of organisms from asymptomatic or convalescent patients.[8]

Principles of the Procedure

Peptone provides amino acids and other nitrogenous substances. Lactose provides a source of energy, and sodium phosphate buffers the medium to maintain the pH. Sodium selenite inhibits gram-positive bacteria and suppresses the growth of most gram-negative enterics other than *Salmonella*. L-cystine is incorporated to improve the recovery of *Salmonella*.[9]

Formula

Difco™ Selenite Cystine Broth

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Lactose	4.0	g
Sodium Phosphate	10.0	g
Sodium Selenite	4.0	g
L-Cystine	0.01	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 23 g of the powder in 1 L of purified water.
2. Heat to boiling. Avoid overheating. DO NOT AUTOCLAVE.
3. Use immediately.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ Selenite Cystine Broth

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	2.3% solution, soluble in purified water upon boiling. Solution is very light amber, clear to very slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Light amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 2.3% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Selenite Cystine Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24 ± 2 hours. After incubation, subculture onto MacConkey Agar plates and incubate plated media at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONIES ON MACCONKEY AGAR
Escherichia coli	25922	10^2-10^3	Partial to complete inhibition	Pink with bile precipitate
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhimurium	14028	10^2-10^3	Good	Colorless
Shigella sonnei	9290	10^2-10^3	Fair to Good	Colorless

Procedure

For feces, food samples or other solid materials, suspend 1-2 g of the specimen in the broth (approximately 10-15% by volume) and emulsify with an inoculating needle, if necessary. Consult references for information about the processing and inoculation of other samples or specimens.[3-7]

Incubate the tubes at 35°C and subculture onto selective and differential media (e.g., MacConkey Agar, XLD Agar, XLT4 Agar, **CHROMagar**™ Salmonella) after 6-8 hours of incubation and again after 12-24 hours of incubation.

Expected Results

After incubation, the number of colonies of pathogens the medium is designed to select should increase. Subculture onto appropriate selective and differential media to isolate pathogens for identification.

Limitations of the Procedure

1. Enrichment broths should not be used as the sole isolation medium. They are to be used in conjunction with selective and nonselective plating media to increase the probability of isolating pathogens, especially when they may be present in small numbers. Consult references for detailed information and recommended procedures.[3,4,6]
2. A brick red precipitate may appear if the medium is overheated during preparation or exposed to excessive moisture during storage.

References

1. Leifson. 1936. Am. J. Hyg. *24*:423.
2. North and Bartram. 1953. Appl. Microbiol. *1*:130.
3. United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.
4. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
5. Horwitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed., vol. 1. AOAC International, Gaithersburg, Md.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
7. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
8. Kelly, Brenner and Farmer. 1985. *In* Lennette, Balows, Hausler and Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
9. Chapin and Murray. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Selenite Cystine Broth

AOAC BAM CCAM COMPF ISO SMD SMWW USP

Cat. No.	268740	Dehydrated – 500 g
	268710	Dehydrated – 2 kg

BBL™ Selenite Cystine Broth

AOAC BAM CCAM COMPF ISO SMD SMWW USP

Cat. No.	297711	Prepared Tubes (A Tubes), 20 mL – Ctn. of 100*

**Store at 2-8°C.*