

YERSINIA SELECTIVE AGAR (7257)

Intended Use

Yersinia Selective Agar is used with cefsulodin and novobiocin for the isolation of *Yersinia enterocolitica*.

Product Summary and Explanation

Yersinia enterocolitica is a significant food or water borne enteric pathogen.^{1,2} Yersinia Selective Agar is a selective and differential medium supporting good growth of *Y. enterocolitica* and some *Yersinia* spp. The formula is based on Cefsulodin-Irgasan-Novobiocin (CIN) Agar developed by Schiemann.³⁻⁶ In comparison with MacConkey Agar and SS Agar, Schiemann found CIN Agar provided increased inhibition of normal enteric organisms and provided improved direct recovery of *Y. enterocolitica* from feces.⁴ Schiemann later modified his formula to further improve growth and recovery of *Y. enterocolitica*.⁶

Principles of the Procedure

Enzymatic Digest of Gelatin, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue provide the nitrogen and amino acids in Yersinia Selective Agar. Yeast Extract is the vitamin source in this formula. Selectivity of Yersinia Selective Agar is due to the presence of Sodium Deoxycholate, Sodium Cholate, Crystal Violet, and Irgasan® that markedly inhibit growth of Gram-positive and many Gram-negative organisms. Supplementation with Cefsulodin and Novobiocin improves inhibition of normal enteric organisms. Differentiation is based on Mannitol fermentation. Organisms capable of fermenting Mannitol lower the pH around the colony, allowing absorption of Neutral Red and turning the colony a red color. Due to the localized pH decrease, a zone of precipitated bile may also be present. Organisms that do not metabolize Mannitol to acid end products will form colorless, translucent colonies. Sodium Chloride maintains the osmotic balance of the medium. Sodium Pyruvate and Magnesium Sulfate stimulate organism growth. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin	17 g
Enzymatic Digest of Casein	1.5 g
Enzymatic Digest of Animal Tissue.....	1.5 g
Yeast Extract.....	2 g
Mannitol	20 g
Sodium Deoxycholate	0.5 g
Sodium Cholate	0.5 g
Sodium Chloride	1 g
Sodium Pyruvate.....	2 g
Magnesium Sulfate	0.01 g
Neutral Red.....	0.03 g
Crystal Violet.....	0.001 g
Irgasan®	0.004 g
Agar	13.5 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Antimicrobial / 10 mL

Cefsulodin	4 mg
Novobiocin	2.5 mg

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 59.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool sterilized medium to 45 - 50°C and aseptically add 10 mL of a sterilized aqueous solution containing 4 mg of Cefsulodin and 2.5 mg of Novobiocin.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and pink-beige to beige.

Prepared Appearance: Prepared medium is medium to dark red-orange to red-purple, and trace to slightly hazy.

Expected Cultural Response: Cultural response on Yersinia Selective Agar incubated at $30 \pm 2^\circ\text{C}$ and examined for growth after 18 - 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reaction
<i>Enterococcus faecalis</i> ATCC® 29212	10^3	Inhibited	N/A
<i>Escherichia coli</i> ATCC® 25922	10^3	Inhibited	N/A
<i>Proteus mirabilis</i> ATCC® 12453	10^3	Inhibited	N/A
<i>Yersinia enterocolitica</i> ATCC® 9610	10 - 300	Growth	Colorless to pink colonies ± bile ppt
<i>Yersinia enterocolitica</i> ATCC® 27729	10 - 300	Growth	Colorless to pink colonies ± bile ppt

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion on the isolation and identification of *Yersinia*, refer to specific procedures.

Results

Yersinia enterocolitica colonies appear translucent or translucent with dark pink centers. Colony edges are entire or irregular. After 48 hour incubation, colonies appear dark pink with a translucent border and may be surrounded by a zone of precipitated bile. Growth of non-*Yersinia* organisms is markedly to completely inhibited.

Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. Further tests are necessary for confirmation of *Yersinia* spp.
2. Some strains of normal enteric organisms may be encountered that are not inhibited or only partially inhibited on complete medium, such as *Citrobacter freundii*, *Serratia liquefaciens*, and *Enterobacter agglomerans*.
3. Growth of *Yersinia frederiksenii*, *Y. kristensenii*, *Y. pseudotuberculosis* and *Y. intermedia* is not inhibited on complete medium. Colonies of these organisms must be differentiated from *Y. enterocolitica* on the basis of additional characteristics.

Packaging

Yersinia Selective Agar	Code No.	7257A	500 g
		7257B	2 kg
		7257C	10 kg

References

1. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Vanderzant, C. and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food. 3rd ed. American Public Health Association, Washington, D.C.
3. **Schiemann, D. A.** 1979. Synthesis of a selective agar medium for *Yersinia enterocolitica*. Can. J. Microbiol. **35**:1298-1304.
4. **Schiemann, D. A.** 1980. *Yersinia enterocolitica*: Observations on some growth characteristics and response to selective agents. Can. J. Microbiol. **26**:1232-1240.
5. **Devenish, J. A., and D. A. Schiemann.** 1981. An abbreviated scheme for identification of *Yersinia enterocolitica* isolated from food enrichments on CIN (cefsulodin-irgasan-novobiocin) agar. Can. J. Microbiol. **27**:937-941.
6. **Schiemann, D. A.** 1982. Development of a two-step enrichment procedure for recovery of *Yersinia enterocolitica* from food. Appl. Environ. Microbiol. **43**:14-27.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.