

# CIN Agar

## Yersinia Selective Agar Base

## Yersinia Antimicrobial Supplement CN

### Intended Use

CIN (cefsulodin-Irgasan<sup>™</sup>\*-novobiocin) Agar supplemented with cefsulodin and novobiocin is a differential and selective medium used in qualitative procedures for the isolation of *Yersinia enterocolitica* from a variety of clinical and nonclinical specimens.

\*Irgasan is a trademark of Ciba-Geigy.

### Summary and Explanation

CIN Agar, also known as Yersinia Selective Agar, was first described by Schiemann as an alternative to MacConkey Agar and other commonly used media for isolation of *Y. enterocolitica*, a causative agent of gastroenteritis.<sup>1</sup> CIN Agar has been found to be far superior to MacConkey, SS, CAL or Y agars for the recovery of *Y. enterocolitica*.<sup>2</sup>

### Principles of the Procedure

Fermentation of mannitol in the presence of neutral red results in a characteristic "bull's-eye" colony, colorless with red center. Selective inhibition of gram-negative and gram-positive organisms is obtained by means of crystal violet, sodium desoxycholate and Irgasan (triclosan). Supplementation with Yersinia Antimicrobial Supplement CN (cefsulodin and novobiocin) improves

inhibition of normal enteric organisms. Organisms that do not metabolize mannitol to acid end products will form colorless, translucent colonies.

### Formulae

#### Difco<sup>™</sup> Yersinia Selective Agar Base

Approximate Formula\* Per Liter

Peptone .....	17.0	g
Proteose Peptone .....	3.0	g
Yeast Extract .....	2.0	g
Mannitol .....	20.0	g
Sodium Pyruvate .....	2.0	g
Sodium Chloride .....	1.0	g
Magnesium Sulfate Heptahydrate .....	10.0	mg
Sodium Desoxycholate .....	0.5	g
Sodium Cholate .....	0.5	g
Irgasan <sup>™</sup> .....	4.0	mg
Agar .....	13.5	g
Crystal Violet .....	1.0	mg
Neutral Red .....	30.0	mg

#### Difco<sup>™</sup> Yersinia Antimicrobial Supplement CN

Formula Per 10 mL Vial

Cefsulodin .....	4.0	mg
Novobiocin .....	2.5	mg

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

### User Quality Control

#### Identity Specifications

##### Difco<sup>™</sup> Yersinia Selective Agar Base

Dehydrated Appearance: Light beige to light pinkish beige, free-flowing, homogeneous.

Solution: 5.95% solution, soluble in purified water upon boiling. Solution is reddish-purple, very slightly to slightly opalescent.

Prepared Appearance: Reddish-orange, very slightly to slightly opalescent.

Reaction of 5.95% Solution at 25°C: pH 7.4 ± 0.2

##### Difco<sup>™</sup> Yersinia Antimicrobial Supplement CN

Dehydrated Appearance: Lyophilized, white, homogeneous cake.

Solution: Soluble on rehydration with 10 mL purified water. Solution is colorless, clear.

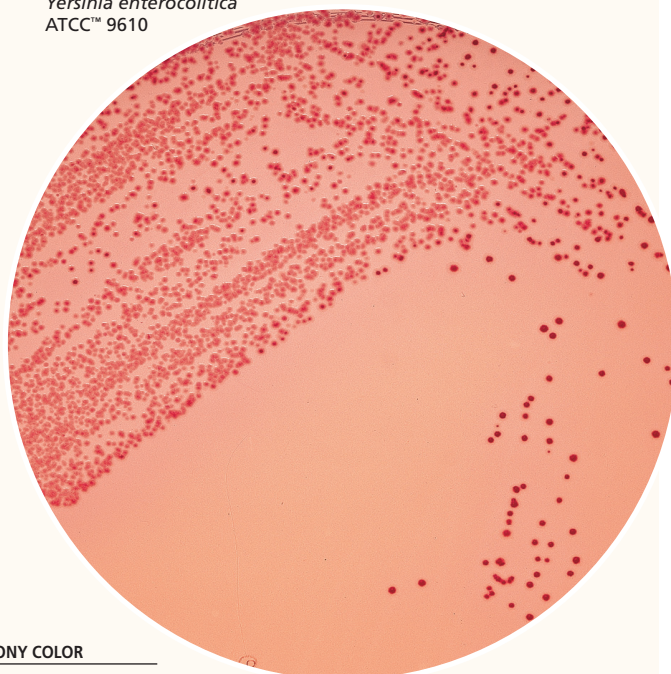
#### Cultural Response

##### Difco<sup>™</sup> Yersinia Selective Agar Base

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC <sup>™</sup>	INOCULUM CFU	RECOVERY	COLONY COLOR
<i>Enterococcus faecalis</i>	29212	10 <sup>3</sup>	Inhibition	—
<i>Escherichia coli</i>	25922	10 <sup>3</sup>	Inhibition	—
<i>Proteus mirabilis</i>	12453	10 <sup>3</sup>	Inhibition	—
<i>Pseudomonas aeruginosa</i>	27853	10 <sup>3</sup>	Inhibition	—
<i>Yersinia enterocolitica</i>	9610	10 <sup>2</sup>	Good	Colorless with dark pink centers, may have bile precipitate

*Yersinia enterocolitica*  
ATCC<sup>™</sup> 9610



## Directions for Preparation from Dehydrated Product

### **Yersinia Antimicrobial Supplement CN**

1. To rehydrate the supplement, aseptically add 10 mL of purified water to the vial.
2. Invert the vial several times to dissolve the powder.

### **Yersinia Selective Agar (CIN Agar)**

1. Suspend 59.5 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. Avoid overheating.
4. For the preparation of CIN Agar, cool to 45-50°C and aseptically add 10 mL of rehydrated Yersinia Antimicrobial Supplement CN. 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 plates at 25°C for 24-48 hours.

If a cold enrichment procedure is desired, inoculate the specimen into phosphate buffered saline and hold at 4°C for up to 21 days.<sup>3,4</sup> Periodically subculture onto plates of CIN Agar, streaking for isolation. Incubate plates as stated above.

## Expected Results

Typical *Y. enterocolitica* colonies will have deep-red centers surrounded by a transparent border giving the appearance of a “bull’s eye.”

Growth of non-*Yersinia* organisms is markedly to completely inhibited.

## Limitation of the Procedure

Although certain strains of *Yersinia* can be recovered by direct plating, others may require cold enrichment (4°C) in phosphate-buffered saline.<sup>3</sup> However, cold enrichment may not be practical because of the long incubation time and because it selects for nonpathogenic *Yersinia* species.<sup>4</sup>

## References

1. Schiemann. 1979. Can. J. Microbiol. 25:1298.
2. Head, Whitty and Ratnam. 1982. J. Clin. Microbiol. 16:615.
3. Weissfeld and Sonnenwirth. 1982. J. Clin. Microbiol. 15:508.
4. Wanger. 2007. In Murray, Baron, Jorgensen, Landry and Pfaller, (ed.), Manual of clinical microbiology. 9th ed. American Society for Microbiology, Washington, D.C.

## Availability

### **Difco™ Yersinia Selective Agar Base**

**BAM COMPF ISO SMD**

Cat. No. 218172 Dehydrated – 500 g

### **Difco™ Yersinia Antimicrobial Supplement CN**

**BAM COMPF ISO SMD**

Cat. No. 231961 Vial – 6 × 10 mL\*

### **BBL™ CIN Agar (Yersinia Selective Agar)**

**BAM BS12 CMPh2 COMPF ISO MCM9 SMD**

*United States and Canada*

Cat. No. 221848 Prepared Plates – Pkg. of 10\*  
299579 Prepared Plates – Ctn. of 100\*

*Europe*

Cat. No. 254056 Prepared Plates – Pkg. of 20\*  
254088 Prepared Plates – Ctn. of 120\*

*Japan*

Cat. No. 251139 Prepared Plates – Pkg. of 20\*

*Mexico*

Cat. No. 230550 Prepared Plates – Pkg. of 10\*

\*Store at 2-8°C.