

95760 Yersinia Selective Agar (CIN Agar; Cefsulodin-Irgasan-Novobiocin Agar)

For the selective cultivation of Yersinia, in particular *Y. enterocolitica* and *Y. pseudotuberculosis* acc. to Schiemann (1979), from clinical samples as well as food, water etc.

Composition:

Ingredients	Grams/Litre
Mixed peptone	20.0
Yeast extract	2.0
D-Mannitol	20.0
Sodium pyruvate	2.0
Sodium chloride	1.0
Magnesium sulfate heptahydrate	0.01
Sodium deoxycholate	0.5
Neutral red	0.03
Crystal violet	0.001
Agar	12.5
Final pH 7.4 \pm 0.2 at 25°C	

Store below 30°C and the prepared medium at 2-8°C. Use before expiry date on the label.

Appearance: Faint yellow, faint beige and faint brown, homogeneous, free flowing powder.

Gelling: Firm

Color and Clarity: light orange to orange and light red to red, clear to slightly opalescent gel form in

Petri plates.

Directions:

Dissolve 58 g in 1 litre distilled water. Sterilize by autoclaving at 121°C for 15 minutes. Cool to ~ 50 °C and add 2 vials of Yersinia selective Supplement (Prod No 75258). Mix well and pour into petri dishes.

Principle and Interpretation:

At the moment, most human illness cases caused by *Yersinia* originate from *Y. enterocolitica*. This organism is the cause of yersiniosis, an infectious disease with symptoms like fever, abdominal pain, and diarrhea. Other clinically important species of this genus are *Y. pseudotuberculosis* (symptoms similar to *Y. enterocolitica* except in most cases

no diarrhea is seen) and *Y. pestis* (organism responsible for the bubonic plague). Most infections are acquired through contaminated food, like raw or undercooked pork products, seafood, vegetables, unpasteurized milk or untreated water. However, infections may also occur after contact with infected animals or faeces, or through transmission by fleas. Yersinia Selective Agar is used to isolate *Y. enterocolitica* and *Y. pseudotuberculosis*, from clinical and non-clinical specimens. The formulation is based on CIN Agar of Schiemann (1, 2) and is recommended by ISO Committee (3). Schiemann (1) modified his previous formula of CIN medium by replacing bile salts with sodium deoxycholate. Sodium pyruvate and magnesium is for the better recovery of the organisms.

Mixed peptones provide carbon, nitrogen, vitamines and other essential growth nutrients. Mannitol fermenting strains, like *Yersina enterocolitica* and *Yersina pseudotuberculosis*, grow as dark pink centre. The red colour is due to production of acid from mannitol, absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8. *Yearsina* shows a turbid zone due to the precipitation of sodium deoxycholate. Mannitol negative organisms form colourless and translucent colonies.



Gram-positive and most gram-negative organisms are inhibited by sodium deoxycholate and crystal violet. Sodium chloride maintains the osmotic equilibrium and agar is the solidifying agents. Addition of the antibiotics cefsulodin, irgasan and novobiocin make the medium highly selective for *Yersinia*. *Aeromonas, Serratia liquefaciens, Citrobacter freundi* and *Enterobacter agglomerans* may resemble *Yersinia* that can be further differentiated by biochemical tests. For the isolation of Yersinia direct plating and pour plating can be taken. Incubate at 22-32°C for 24-48 hours or suspend the sample (food, faeces, etc.) in sterile Phosphate Buffer Saline and incubate for up to 21 days (4) at 4°C. Periodically subculture samples onto Yersinia Agar Plate and incubate as above.

Cultural characteristics after 24 - 48 hours at 22-32°C.

Organisms (ATCC)	Inoculu m [CFU]	Growth	Recovery [%]	Colony appearance
Escherichia coli (25922)	≥10 ³	+	0	-
Enterococcus faecalis (29212))	≥10 ³	+	0	-
Proteus mirabilis (25933)	≥10 ³	+	0	-
Pseudomonas aeruginosa (27853)	≥10 ³	+	0	-
Yersina enterocolitica (27729)	50-100	+++	≥50	transluscent with dark pink centre & bile precipitate.

References:

- 1. D.A. Schiemann, Can. J. Microbiol., 25, 1298. (1979)
- 2. D.A. Schiemann, Can. J. Microbiol., 26, 1232 (1980)
- 3. International Organization for Standardization (ISO), Draft ISO/DIS 10273:1994
- 4. Weissfeild and Sonnenwirth, J. Clin. Microbiol. 15, 508 (1982)

Precautions and Disclaimer

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