YERSINIA SELECTIVE AGAR (CIN)

INTENDED USE
Remel Yersinia Selective Agar (CIN) is a solid medium recommended for selective and differential isolation of *Yersinia enterocolitica* from clinical specimens and food.

SUMMARY AND EXPLANATION
In 1979, Schiemann described a selective and differential medium for isolation of *Y. enterocolitica* which contained cefsulodin, Irgasan®, novobiocin, bile salts, and crystal violet as selective agents.[1] He later modified the formula by substituting sodium desoxycholate for bile salts and reduced novobiocin to 2.5 mg/liter, further improving growth and recovery of *Y. enterocolitica*.[2] In a study comparing selective and differential media for isolation of enteric pathogens, Yersinia Selective Agar was found to be superior to other media (e.g., SS (Salmonella-Shigella), MacConkey, CAL (cellobiose, arginine, lysine), and Y agars) for isolation of *Yersinia* spp.[3] Some strains of *Yersinia* may require cold enrichment (4°C) in phosphate buffered saline prior to inoculation of Yersinia Selective Agar.[4] Yersinia Selective Agar complies with the specifications of the AOAC International.[5]

PRINCIPLE
Peptones provide nitrogen, amino acids, and peptides necessary for bacterial growth. Sodium chloride is a source of essential electrolytes and maintains osmotic equilibrium. Yeast extract provides B-complex vitamins. Mannitol is the carbohydrate which, in combination with neutral red dye, enables differentiation of enteric gram-negative bacilli. Organisms which ferment mannitol produce a localized pH drop around the colony which is followed by absorption of the neutral red dye. This results in a characteristic "bulls-eye" colony which has a colorless, translucent border and a red center. Colonies of *Y. enterocolitica* typically develop a "bulls-eye" appearance after 24-48 hours incubation at 25°C. Crystal violet, sodium desoxycholate, cefsulodin, Irgasan® (triclosan), and novobiocin are selective agents which inhibit *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*, as well as gram-positive organisms.

REAGENTS (CLASSICAL FORMULA)*

Mannitol	20.0 g	Neutral Red	30.0 mg
Peptone	10.0 g	Cefsulodin	4.0 mg
Beef Extract	5.0 g	Irgasan®	4.0 mg
Meat Peptone	5.0 g	Novobiocin	2.5 mg
Sodium Pyruvate	2.0 g	Crystal Violet	1.0 mg
Yeast Extract	2.0 g	Magnesium Sulfate	1.0 mg
Sodium Chloride	1.0 g	Agar	12.0 g
Sodium Desoxycholate	0.5 g	Demineralized Water	1000.0 ml

pH 7.4 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Inoculate the specimen as soon as possible after it is received in the laboratory.
2. If the material is being cultured directly from a swab, roll the swab over a small area of the agar surface and streak for isolation.
3. Incubate plates aerobically at 25°C for 24-48 hours.
4. If cold enrichment is preferred, inoculate the specimen into Phosphate Buffered Saline (PBS) (REF R062582 or a suitable alternative) and hold at 4°C for up to 21 days. Periodically subculture PBS onto plates of Yersinia Selective Agar, following established laboratory guidelines. Incubate plates aerobically at 25°C for 24-48 hours.
5. Examine plates for typical colonies of *Y. enterocolitica* which develop a dark red "bulls-eye" center with a translucent border. Further biochemical and/or serological testing may be required to definitively identify *Y. enterocolitica*. Consult appropriate references for further instructions.[9]

Pour Tube: Melt Yersinia Selective Agar pour tube (REF R09999) in a boiling water bath and cool to 45-50°C. Add rehydrated CN Selective Supplement (REF R45050). Mix and dispense into a sterile petri dish and proceed with the instructions above.

QUALITY CONTROL
All lot numbers of Yersinia Selective Agar (CIN) have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing meets or exceeds CLSI standards.[6] Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
**Yersinia enterocolitica* ATCC® 9610	Aerobic, 24-48 h @ 25°C	Growth, clear colonies w/ deep red center
**Enterococcus faecalis* ATCC® 29212	Aerobic, 24-48 h @ 25°C	Inhibition (partial to complete)
**Escherichia coli* ATCC® 25922	Aerobic, 24-48 h @ 25°C	Inhibition (partial to complete)
Proteus mirabilis ATCC® 12453	Aerobic, 24-48 h @ 25°C	Inhibition (partial to complete)
**Pseudomonas aeruginosa* ATCC® 27853	Aerobic, 24-48 h @ 25°C	Inhibition (partial to complete)

*CLSI recommended organism

LIMITATIONS
1. Gram-negative bacilli other than *Y. enterocolitica* grow on Yersinia Selective Agar, including *Aeromonas, Serratia, Enterobacter,* and *Citrobacter*. These organisms cannot be differentiated from *Y. enterocolitica* on the basis of colony morphology alone. Further biochemical and/or serological testing is required for definitive identification of *Y. enterocolitica*.[7,8]

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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