Yersinia Selective Supplement

PRODUCT INFORMATION

C058-100mg - Cefsulodin Sodium, Powder, 100 mg

C058-250mg - Cefsulodin Sodium, Powder, 250 mg

C058-1g - Cefsulodin Sodium, Powder, 1g

DESCRIPTION

Yersinia Selective Agar Base with Yersinia Selective Supplement is a selective medium for *Yersinia enterocolitica* when used with Yersinia Selective Supplement (Schiemann CIN Medium).

BACKGROUND

Cefsulodin is the third generation cephalosporin antibiotic that has very specific activity against Pseudomonas aeruginosa. It has no significant activity against other Gram-negative bacteria and very limited activity against Gram-positive bacteria and anaerobic bacteria.

Cefsulodin was first synthesized and patented by Takeda Pharmaceutical Company in 1977. In 2002, Takeda stopped production of Cefsulodin. Many years of low-stability cefsulodin production has led to a widespread reduction of laboratory and research usages. Current attempts (i.e. IDEXX Laboratories, Inc.) of increasing purity and stability of cefsulodin center around recrystallization. Typically the process entails the following: Cefsulodin is dissolved in an organic solvent, sodium, water or any mixture thereof, then subsequently recrystallized through separation of the unwanted fraction. .

Recently, TOKU-E has found that the main cause of cefsulodin instability stems from one key impurity in 7-ACA (7-aminocephalosporanic acid- a raw material used in the synthesis of cefsulodin). In order to produce high-purity, high-stability cefsulodin, TOKU-E uses industrial HPLC to remove significant quantities of this impurity in 7-ACA and thus produce ultra-pure, ultra-stable, and ultra-potent cefsulodin.

Novobiocin is an aminocoumarin antibiotic that is produced by the actinomycete *Streptomyces niveus*, which has recently been identified as a subjective synonym for *S. spheroides* a member of the order Actinobacteria

Mechanism of action

The molecular basis of action of novobiocin, and other related drugs clorobiocin and coumermycin A1 has been examined. Aminocoumarins are very potent inhibitors of bacterial DNA gyrase and work by targeting the GyrB subunit of the enzyme involved in energy transduction. Novobiocin as well as the other aminocoumarin antibiotics act as competitive inhibitors of the ATPase reaction catalysed by GyrB. The potency of novobiocin is considerably higher than that of the fluoroquinolones that also target DNA gyrase, but at a different site on the enzyme. The GyrA subunit is involved in the DNA nicking and ligation activity.

APPLICATION IN YERSINIA SELECTIVE AGAR BASE

Yersinia Selective Medium (CIN Medium) is based on the formulation of Schiemann and is recommended for the isolation and enumeration of *Yersinia enterocolitica* from clinical specimens and food.

Yersinia enterocolitica is becoming increasingly recognised as a cause of diarrhoeal disease of man. Infection by the organisms results in diarrhoea, malaise, nausea and fever, plus constant abdominal pain over a period of 1-2 days. The organism has also been shown as a cause of polyarthritis, mesenteric adenitis and septicaemia. It is likely that human infections are directly or indirectly derived from animal sources and may be contracted through the ingestion of contaminated food. Initially serotypes 0:3 and 0:9 were implicated in human infections but since then other serotypes, mainly 0:5 and 0:8 have also been involved. It is important to note that incidence of disease caused by the various serotypes of *Yersinia enteroccolitica* is currently reported to vary considerably with geographical location. It is expected that with provision of a selective medium, a higher isolation rate will result, and *Yersinia enterocolitica* will be recognised as more common and widespread than previously suspected.

Yersinia Selective Agar Base and the selective supplement have been developed specifically for the optimum growth and recovery of *Yersinia enterocolitica* after 18-24 hours incubation at 32°C. Schiemann modified his earlier formulation for CIN Medium by replacing bile salts with sodium desoxycholate (0.5 g/l) and by reducing the concentration of novobiocin from 15 to 2.5 mg/l in order to eliminate the inhibition of some strains of serotype 0:8.

The typical colonies of Yersinia enterocolitica will develop as a red bull's-eye surrounded by a transparent border and will vary considerably among serotypes in colony size, smoothness and the ratio of the border to centre diameter. Most other organisms that are capable of growing will produce larger colonies (>2 mm in diameter) with diffuse pinkish centres and opaque outer zones. *Serratia liquefaciens, Citrobacter freundii* and *Enterobacter agglomerans* may give a colonial morphology resembling *Yersinia enterocolitica*. These organisms can be differentiated from *Yersinia enterocolitica* by biochemical tests.

Test for growth on Nutrient and MacConkey Agars, test for indole and urease production and for acid reactions from sucrose, cellobiose, amygdalin, melibiose, rhamnose and reffinose. Carry out tests at 30°C rather than 37°C.

Content concentrations

Typical Formula*	mg/litre
Yersinia Selective Agar Base	
Special peptone	20
Yeast extract	2
Mannitol	20
Sodium pyruvate	2
Sodium chloride	1
Magnesium sulphate	0.01
Sodium desoxycholate	0.5
Neutral red	0.03
Crystal violet	0.001
Agar	12.5
Final pH 7.4 ± 0.2 @ 25°C	
Yersinia Selective Supplement	
Cefsulodin	15
Irgasan	4
Novobiocin	2.5
* Adjusted as required to meet performance standards	

Table 1 - Typical Formula for Yersinia Selective Agar Base and Yersinia Selective Supplement

METHOD

Preparation

Suspend appreciate amount of Yersinia Selective Supplement in distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Allow to cool to approximately 50°C and aseptically add the contents of Yersinia Selective Supplement reconstituted as directed in the instructions for use that accompany the product. Mix gently and pour into sterile Petri dishes.

Protocol

Direct plate method

1. Pour plates of Yersinia Selective Agar and dry the surface.

2. Inoculate the plates with a suspension of the food, faeces, etc. to produce single colonies.

3. Incubate at 32°C for 24 hours.

Cold Enrichment in Phosphate Buffered Saline

1. Inoculate food, faeces, etc., into M/15 phosphate buffered saline.

2. Hold at 4°C for up to 21 days.

3. Periodically sub-culture samples on to plates of Yersinia Selective Agar.

4. Incubate at 32°C for 24 hours.

CIN Agar had been used for isolation of *Leptospira* spp. With enhancement of its nutritional properties and addition of 5-fluorouracil to increase selectivity it has also been used to demonstrate the presence of Arcobacter spp. in ground pork.

Colonial morphology

Typical colonies of Yersinia enterocolitica will develop a red bull's-eye surrounded by a transparent border. The colony size, smoothness and the ratio of the border to centre diameter will vary considerably among serotypes.

Identification of isolates

The presumptive colonies are confirmed as *Yersinia enterocolitica* by the biochemical reactions.

1. Growth at 4°C and on Nutrient/MacConkey Agars.
2. Motile at 22°C
3. Indole production variable
4. Urease positive
5. Ornithine decarboxylase positive
6. Acid production from sucrose, cellobiose, amygdalin, rhamnose and raffinose
7. No acid production from melibiose

Quality control

Positive control:

Yersinia enterocolitica ATCC® 27729: Good growth; transparent, red, bull's-eye colonies

Negative control:

Escherichia coli ATCC® 25922: Inhibited

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