HIMEDIA®

Technical Data

Cefsulodin, Irgasan™ (Triclosan)and Novobiocin (CIN) Agar M843I

Intended use

Recommended for the selective isolation and enumeration of *Yersinia enterocolitica* from food samples. The composition and performance criteria of this medium are as per the specifications laid down in ISO 10273:2017(E)

Composition**

ISO 10273:2017(E)

Ingredients	g / L
Enzymatic digest of gelatin	17.000
Enzymatic digest of casein and animal tissues	3.000
Yeast extract	2.000
Mannitol	20.000
Sodium pyruvate	2.000
Sodium chloride	1.000
Magnesium sulfate heptahydrate (MgSO4 7H2O)	0.010
Sodium desoxycholate	0.500
Neutral red	0.030
Crystal violet	0.001
Agar	12.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Cefsulodin, Irgasan™ (Triclosan)and Novobiocin Agar

Ingredients	g / L
Gelatin Peptone	17.000
Mixture of Peptone and Tryptone#	3.000
Yeast extract	2.000
Mannitol	20.000
Sodium pyruvate	2.000
Sodium chloride	1.000
Magnesium sulphate	0.010
Sodium deoxycholate	0.500
Neutral red	0.030
Crystal violet	0.001
Agar	12.000
Final pH (at 25°C)	7.4±0.2

- Enzymatic digest of casein and animal tissues

Supplements to be added after autoclaving	g / L
Cefsulodin	15mg
Triclosan(Irgasan)	4mg
Novobiocin	2.5mg

FD034-CTN Selective Supplement	1 Vial
Cefsulodin	7.500mg
Triclosan(Irgasan)	2mg
Novobiocin	1.250mg

Directions

Suspend 29.02 grams in 500 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add reconstituted contents of 1 vial of CTN Selective Supplement (FD034). Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Yersinia enterocolitica is widely distributed in lakes and reservoirs. Epizootic outbreaks of diarrhea, lymphadenopathy, pneumonia and spontaneous abortions occur in various animals. It is the most common species of *Yersinia* recovered from clinical specimens. *Y. enterocolitica* is biochemically more active at room temperature than at 37°C. Yersinia Selective Agar Base with added Yersinia Selective Supplement is used to isolate *Y.enterocolitica* 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. The medium differentiates between mannitol fermenting and non-fermenting bacteria. Microorganisms that ferment the sugar mannitol acidify the medium and cause a localized drop in pH around the colonies. In presence of neutral red, the colonies take red colour. Mannitol negative organisms form colourless and translucent colonies. The medium is selective due to the presence of sodium deoxycholate and crystal violet, which inhibit gram-positive and a number of gram-negative bacteria. Addition of antibiotic supplement makes it highly selective for *Yersinia.* Typical colonies of *Y. enterocolitica* will form dark red colonies resembling bull's eye, which are normally surrounded by a transparent border. Colony size, smoothness and ratio of the border to center diameter may vary among different serotypes. For the isolation of *Y. enterocolitica* by direct plating and pour plating, inoculate the specimen directly onto the medium. Incubate at 22-32°C for 24-48 hours or suspend the sample (food, faeces, etc.) in sterile Phosphate Buffer Saline and incubate for upto 21 days (4) at 4°C. Periodically subculture samples onto Yersinia Agar Plate and incubate as above.

Type of specimen

Food samples

Specimen Collection and Handling:

Processing (3,4)

Enrichment : For the first initial suspension place the sample (x) in known volume of the PSB broth (M941I), to give a dilution of 1/10 dilution (by mass/volume or volume/volume). Homogenize the suspension using a peristaltic blender for 2 min. Incubate at 22°C to 25°C for 2 to 3 days with or 5 days without agitation.

For the second inition suspension in the same way with the ITC broth (M1220) so as to obtain a test portion/enrichment medium dilution of 1/100 (mass/volume or volume/volume). Incubate at 25°C for 48 hours.

Isolation : 1. Inoculate the culture obtain from PSB culture on the surface of CIN agar plate and incubate at 30°C for 24 to 48 hours.

2.Alkaline treatment : Using sterile pipette transfer 0.5ml of the PSB culture into 4.5 ml of KOH solution and mix for 20 seconds only. Immediately inoculate on CIN agar plate. Incubate at 30°C for 24 to 48 hours.

3.Using ITC culture inoculate the surface of SSDC agar plate (M1703). Incubate at 30°C for 24 to 48 hours.

4.Purification : Streak the selected colonies on the surface of Nutrient Agar (M561A). Incubate at 30°C for 24 hours.

5.Confirmation : Streak the slant of the agar and incubate at 30°C for 24 hours. A black halo around the colonies obtain positive reaction.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. *Serratia liquefaciens, Citrobacter freundi* and *Enterobacter agglomerans* may resemble *Y.enterocolitica* that can be further identified by biochemical tests.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.20% Agar gel.

Colour and Clarity of prepared medium

Orange red coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 5.8% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Productivity : Cultural characteristics observed with added CTN Selective Supplement (FD034) after an incubation at 30±1° C for 24±2hours.

Selectivity: Cultural characteristics observed with added CTN Selective Supplement (FD034) after an incubation at 30±1°C for 24±2hours.

Organism	Inoculum (CFU)	Growth	Characteristic reaction of target organism on CIN Agar (M843I)
Productivity			
Yersinia enterocolitica subsp. *palearctica* serotype O:3 NCTC 13769 (00126)*	50-100	>10 colonies	Transparent or translucent circular, smooth colonies with deep red sharp bordered centre.
Yersinia enterocolitica subsp. *palearctica* serotype O:3 NCTC 13769 (00126)*	50-100	>10 colonies	Transparent or translucent circular, smooth colonies with deep red sharp bordered centre.

Selectivity		
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1.Schiemann D. A., 1979, Can. J. Microbiol., 25: 1298.
2.Schiemann D. A., 1980, Can. J. Microbiol., 26: 1232.
3.International Organization for Standardization (ISO), 1994 Draft ISO/DIS 10273.
4.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.
5.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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