

**INSTRUCTIONS FOR USE**

CIN AGAR BASE YERSINIA SELECTIVE SUPPLEMENT

Dehydrated culture medium and selective supplement



Yersinia enterocolitica on CIN Agar

1 - INTENDED USE

In vitro diagnostic. Selective and differential basal medium and selective supplement for the isolation and characterisation of *Y. enterocolitica* from clinical and other specimens.

2 - COMPOSITION

CIN AGAR BASE

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Peptone	20.000 g
Yeast extract	2.000 g
Mannitol	20.000 g
Sodium pyruvate	2.000 g
Sodium chloride	1.000 g
Magnesium sulphate	0.010 g
Sodium desoxycholate	0.500 g
Irgasan	0.004 g
Neutral red	0.030 g
Crystal violet	0.001 g
Agar	12.000 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

YERSINIA SELECTIVE SUPPLEMENT

(VIAL CONTENTS FOR 500 mL OF MEDIUM)

Cefsulodin	7.50 mg
Novobiocin	1.25 mg

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The genus *Yersinia* includes 20 species, among them, only *Y. pestis*, *Y. pseudotuberculosis*, and certain strains of *Y. enterocolitica* are of pathogenic importance for humans, whereas the other species are of environmental origin. Of these, *Y. enterocolitica* is the most important as a cause of foodborne illness. *Y. enterocolitica* is a Gram-negative bacillus, motile at temperatures of 22–29°C but non-motile at 37°C. The most common form of disease due to *Y. enterocolitica* is gastroenteritis associated with consumption of contaminated food or water.¹ *Y. enterocolitica* is a heterogeneous group of strains, which are traditionally classified by biotyping into six biogroups on the basis of phenotypic characteristics, and by serotyping into more than 57 O serogroups, on the basis of their O (lipopolysaccharide or LPS) surface antigen. Of the six biotypes, five are recognised to be pathogenic (1B, 2-5). The most important *Y. enterocolitica* serogroup in many European countries is serogroup O:3 followed by O:9, whereas the serogroup O:8 is mainly detected in the United States.² Cefsulodin-Irgasan-Novobiocin (CIN) Agar, originally developed in 1979 by Schiemann,³ is a selective and differential medium for the isolation and characterisation of *Y. enterocolitica* from clinical^{1,2} and non-clinical specimens^{4,5}. The medium is recommended by ISO 10273⁴ and by FDA-BAM⁵ for the detection of *Y. enterocolitica* in food.

Compared with MacConkey agar, CAL agar and Y medium, CIN agar has been found to be the most effective medium for the recovery of *Y. enterocolitica*, inhibiting almost completely the faecal flora, while at the same time supporting luxuriant growth of *Y. enterocolitica*.⁶ The medium is highly selective: Schiemann⁶ and Devenish⁷ reported that only some strains of *C. freundii*, *S. liquefaciens* and *E. agglomerans* grow on CIN Agar; the colonies of these contaminants have an appearance similar to *Y. enterocolitica*.

Peptone and yeast extract provide nutrients for bacterial growth. Gram-positive and some Gram-negative bacteria (e.g. *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*) are inhibited by the selective agents present in the medium base (sodium desoxycholate, crystal violet, irgasan) and in the lyophilized supplement (cefsulodin and novobiocin). Mannitol is present as a fermentable carbohydrate: mannitol fermenting bacteria induce acidification of the medium with precipitation of desoxycholate and absorption of neutral red; *Y. enterocolitica* therefore cultivates with the characteristic aspect of the "bull's eye" colonies: the center of the colony deep red with a transparent margin. Organisms that do not metabolize mannitol to acid end-products will form colourless colonies.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 28.75 g in 500 mL of cold purified water. Heat to boiling stirring constantly, sterilize by autoclaving at 121°C for 15 minutes. Cool to 47–50°C and add the contents of one vial of Yersinia Selective Supplement reconstituted with 5 mL of sterile purified water under aseptic conditions. Mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

CIN Agar Base

Dehydrated medium appearance	pinkish-beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-violet, limpid
Final pH at 20-25 °C	7.4 ± 0.2

Yersinia Selective Supplement

Freeze-dried supplement appearance	short, dense, white pastille
Reconstituted supplement appearance	limpid, colourless





6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
CIN Agar Base	Dehydrated medium	4013022	500 g (8,7 L) CND: W0104010101; EDMA: 14.01.01.01; RDM: 1858137 /R
Yersinia Selective Supplement	Freeze-dried supplement	4240011	10 vials, each for 500 mL of medium CND: W0104010104; EDMA: 14.01.01.04; RDM: 1892760/R

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, incubator and laboratory equipment as required, sterile loops and swabs, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

CIN Agar is intended for the bacteriological processing of clinical specimens such as faeces and rectal swab^{1,2} and non-clinical specimens such as food and animal feeding stuffs^{4,5}. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.⁸ Collect specimens before antimicrobial therapy where possible. Consult appropriate standard methods for details on food sample collection and preparation.^{4,5}

9 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

Clinical specimens

Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Faeces may be diluted 1:4 in sterile saline solution or 0.1% peptone water. It has been shown that dilution significantly reduces the amount of competing flora without compromising isolation of low numbers of pathogens.²

Incubate aerobically at 28-30°C for 24-48 hours.²

Food chain samples⁴

The general procedure involves:

- Direct plating of sample suspension prepared in PSB broth* on CIN Agar plate and incubation at 30°C ± 1°C for 24 h ± 2 h, or
- Enrichment in PSB Broth and in ITC broth** with incubation at 25°C ± 1°C for 44 h ± 4, followed by alkaline treatment of the cultures (0.5 mL of culture + 4.5 mL KOH 0.5% in saline solution for 20 s ± 5 s) and plating onto CIN Agar (incubation at 30°C ± 1°C for 24 h ± 2 h).
- A second plating medium may be chosen by the user (e.g. Chromogenic Yersinia Agar)***

Notes

* Yersinia PSB Broth (REF 402270). ** Yersinia ITC Broth Base REF 402265 added with Potassium Chlorate Supplement (REF 4240065) and Ticarcillin Irganon Antimicrobial Supplement (REF 4240060). *** Chromogenic Yersinia Agar Base (REF 408050) added with Chromogenic Yersinia Supplement (REF 4240095).

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

Y. enterocolitica will ferment the mannitol and will develop colonies with deep red centres with sharp borders (irregular or entire), surrounded by an outer zone which is usually translucent ("bull's eye" colonies). The colony size, smoothness and the ratio of the border to centre diameter will vary considerably among serotypes.

Mannitol non fermenters will grow with colourless or pale yellow colonies.

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

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, it is responsibility of the end-user to perform Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.⁹

CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
<i>Y. enterocolitica</i> ATCC 9610	28-30°C / 18-24H / A	good growth, colonies with red centre
<i>P. aeruginosa</i> ATCC 27853	28-30°C / 44-48H / A	growth partially inhibited, colourless colonies
<i>E. coli</i> ATCC 25922	28-30°C / 44-48H / A	inhibited
<i>E. faecalis</i> ATCC 19433	28-30°C / 44-48H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated CIN Agar Base, supplemented with Yersinia Selective Supplement, is tested for productivity and selectivity by comparing the results with previously approved Reference Batches.

Productivity is tested by a quantitative test with the target strains *Y. enterocolitica* ATCC 23715 and *Y. enterocolitica* ATCC 9610; plates are inoculated with decimal dilutions in saline of the colonies suspensions and incubated aerobically at 29-31°C for 18-24 hours. The colonies are enumerated on Test Batch (TB) and Reference Batch (RB) and the productivity ratio ($Pr = CFU_{TB} / CFU_{RB}$) is calculated. If Pr is $\geq 0,7$ and if the colonies show typical characteristics ("bull's eye" colonies), the results are considered acceptable and conform to the specifications.

Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target strains *S. marcescens* ATCC 8100, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25823, *E. faecalis* ATCC 29212. *S. marcescens* is partially inhibited, the growth of other non-target strains is totally inhibited at the dilution 10⁻¹.

13 - LIMITATIONS OF THE METHOD





- In case of dense growth of background flora on the CIN plates, the colony size of pathogenic *Y. enterocolitica* can be smaller and the typical red centre can be unclear or absent.⁴
- *Y.intermedia*, *Y.frederiksenii*, and *Y.kristensenii* grow equally as well as *Y.enterocolitica* on CIN Agar and exhibit the same colony morphology.¹⁰
- *Serratia*, *Enterobacter* and *Citrobacter* are poorly inhibited. *Serratia* and *Enterobacter* develop raised and mucoid colonies with a diffuse pink pigmentation, although, occasionally, they can be confused with *Y.enterocolitica* colonies; *Citrobacter* colonies are the closest in appearance to *Yersinia* and cannot be distinguished only by their morphological characteristics.¹⁰
- The majority of *Y.pseudotuberculosis* strains are inhibited by the concentration of 15 mg/L of cefsulodin.¹¹
- Some strains of *Y.enterocolitica* serovar O3 fail to grow on CIN Agar.¹¹
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- The culture medium and the supplement are intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplement are qualitative *in vitro* diagnostics, for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement shall be used in association according to the described directions.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Before use, consult the Material Safety Data Sheets.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our products for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

- **Dehydrated medium:** upon receipt, store at 10-30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use the container must be tightly closed. Discard the product if the container and/or the cap were damaged or in case of evident deterioration of the powder (colour changes, hardening, presence of large lumps).
- **Selective supplement:** upon receipt store at 2-8°C until the expiry date. Do not use beyond this date. Once opened and reconstituted, the vial contents must be used immediately.

16 - REFERENCES

1. Petersen MJ, Gladney LM, Schriefer ME. *Yersinia*. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology, 11th ed. Washington, DC: American Society for Microbiology; 2015.
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3. Schiemann D A. Synthesis of a selective agar medium for *Yersinia enterocolitica*. Can J Microbiol 1979; 25(11):1298-1304.
4. ISO 10273:2017 Microbiology of the food chain -- Horizontal method for the detection of pathogenic *Yersinia enterocolitica*
5. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 8: *Yersinia enterocolitica*. Rev 10/2017
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7. Schiemann DA. Development of a Two-Step Enrichment Procedure for Recovery of *Yersinia enterocolitica* from Food. Appl Environ Microbiol 1982; 43 :14-27
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10. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
11. Fukushima H, Gomyoda M. Growth of *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* Biotype 3B Serotype O3 Inhibited on Cefsulodin-Irgasan-Novobiocin Agar. J Clin Microbiol 1986, 24:116-120

4240011 YERSINIA SELECTIVE SUPPLEMENT



Mixture with hazardous ingredients: cefsulodin sodium and novobiocin
Classification according to Regulation (EC) No 1272/2008

Skin irritation (Category 2)
 Eye irritation (Category 2)
 Skin sensitization (Category 1)
 Specific target organ toxicity - single exposure (Category 3)

Labelling according Regulation (EC) No 1272/2008

Pictogram



Signal word Warning

Hazard statement(s)

H315
 H317
 H319
 H335

Causes skin irritation.
 May cause an allergic skin reaction.
 Causes serious eye irritation.
 May cause respiratory irritation.

Precautionary statement(s)

P261
 P280
 P305 + P351 + P338

Avoid breathing dust.
 Wear protective gloves.
F IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing..

TABLE OF APPLICABLE SYMBOLS FOR DEHYDRATED MEDIUM















REF or REF Catalogue number	LOT Batch code	IVD <i>In vitro</i> Diagnostic Medical Device	 Manufacturer	 Use by
 Temperature limitation	 Contents sufficient for <n> tests	 Consult Instructions for Use	 Keep away from direct light	 Store in a dry place

TABLE OF APPLICABLE SYMBOLS FOR SELECTIVE SUPPLEMENT

REF or REF Catalogue number	LOT Batch code	IVD <i>In vitro</i> Diagnostic Medical Device	 Manufacturer	 Use by
 Temperature limitation	 Contents sufficient for <n> tests	 Consult Instructions for Use	 Store away from direct light	 Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/09

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

