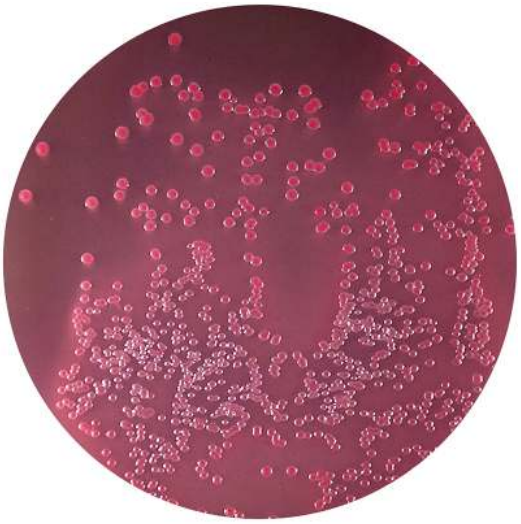
INSTRUCTIONS FOR USE

YERSINIA SELECTIVE AGAR

Ready-to-use plates



Yersinia enterocolitica on CIN Agar

1 - INTENDED USE

In vitro diagnostic device. Selective and differential medium for the isolation and characterisation of *Yersinia enterocolitica* from clinical and other specimens.

2 - COMPOSITION TYPICAL FORMULA *

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 deoxycholate	0.500 g
Irgasan	0.004 g
Neutral red	0.030 g
Crystal violet	0.001 g
Agar	12.000 g
Cefsulodin	15.0 mg
Novobiocin	2,5 mg
Purified water	1000 mL

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

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]
Yersinia selective agar, known also as 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 10273[4] and by FDA-BAM[5] for the detection of *Y.enterocolitica* in food.
Compared with MacConkey agar, CAL agar and Y medium, Yersinia selective agar (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: Schiemann[6] and Devenish[7] 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: sodium deoxycholate, crystal violet, irgasan, cefsulodin and novobiocin. Mannitol is present as a fermentable carbohydrate: mannitol fermenting bacteria induce acidification of the medium with precipitation of deoxycholate and absorption of neutral red; *Y.enterocolitica* therefore cultivates with the characteristic aspect of the "bull's eye" colonies: the centre of the colony deep red with a transparent margin. Organisms that do not metabolize mannitol to acid end-products will form colourless colonies.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	red-violet, limpid
Final pH at 20-25°C	7.4 ± 0.2

5- MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Yersinia selective agar	Ready-to-use plates	549997	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Incubator and laboratory equipment as required, sterile loops and swabs, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Yersinia selective 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]

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8 - 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 29-31°C for 24-48 hours.

Food chain samples[4]

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% 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 Irgasan Antimicrobic Supplement (REF 4240060). *** Chromogenic Yersinia Agar Base (REF 408050) added with Chromogenic Yersinia Supplement (REF 4240095).

9 - 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.

10 - 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, the end user can perform its own Quality Control 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
Y.enterocolitica	ATCC	9610	29-31°C / 18-24H / A	good growth, colonies with red centre
P.aeruginosa	ATCC	27853	29-31°C / 44-48H / A	inhibited
E.coli	ATCC	25922	29-31°C / 44-48H / A	inhibited
E.faecalis	ATCC	19433	29-31°C / 44-48H / A	inhibited

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

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of ready-to-use plates of Yersinia selective agar and of the raw materials used for the production of prepared plates (dehydrated CIN Agar Base, supplemented with Yersinia Selective Supplement), are tested for productivity and selectivity by comparing the results with previously approved Reference Batches.

Productivity is tested by a quantitative test with the target strain *Y.enterocolitica* ATCC 23715; 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 ≥ 0,7 and if the colonies show typical characteristics ("bull's eye" colonies), the results are considered acceptable and conform to the specifications.

Moreover productivity is evaluated by semi-quantitative ecometric technique with the target strains *Y.enterocolitica* ATCC 9610 and *Y.enterocolitica* DSM 13030. After incubation at 29-31°C for 18-24 h in aerobic atmosphere, the extent of growth and morphology of the colonies are evaluated and if they are comparable in both tested batches, the results are judged to comply with 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~~ 25922, *P.aeruginosa* ATCC 27853, *S.aureus* ATCC 25923, *E.faecalis* ATCC 29212. *S.marcescens* is partially inhibited, the growth of other non-target strains is totally inhibited.

12 - 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.[1,11]
- 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.
- This culture medium is 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.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- 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 ready-to use plates 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 culture medium or microbial agents.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product 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 product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

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10. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
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TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2020/09
Instructions for Use (IFU) - Revision 2	Removal of obsolete classification	2023/03

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

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