INSTRUCTIONS FOR USE

YERSINIA SELECTIVE SUPPLEMENT

Freeze-dried selective supplement

1 - INTENDED USE

In vitro diagnostic. Mixture of antimicrobials to be added to CIN Agar Base for the for the isolation and characterisation of *Y.enterocolitica* from clinical and other specimens.

2 - COMPOSITION - 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

Yersinia Selective Supplement is a freeze-dried mixture of antimicrobials to be used as a supplement of CIN Agar Base for the isolation and characterisation of *Y.enterocolitica*.

The complete medium Cefsulodin-Irgasan-Novobiocin (CIN) Agar, originally developed in 1979 by Schiemann,[1] is a selective and differential medium for the isolation and characterization of Y. enterocolitica from clinical[2,3] and non-clinical[4,5] specimens. The medium is recommended by ISO 10273[4] and by FDA-BAM[5] for the determination of Y.enterocolitica in food.

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 deoxycholate, crystal violet, irgasan) and in the lyophilized supplement (cefsulodin and novobiocin).

4- DIRECTIONS

Aseptically reconstitute the contents of one vial with 5 mL of sterile purified water and mix gently to dissolve.

Prepare 500 mL of CIN Agar Base (REF 401302), autoclaved and cooled to 47-50°C and add the contents of one vial of Yersinia Selective Supplement under aseptic conditions. Mix well and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

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

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Yersinia Selective Supplement	Freeze-dried supplement	4240011	10 vials, each for 500 mL of medium

7 - MATERIALS REQUIRED BUT NOT PROVIDED

CIN Agar Base (REF 401302), 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 Base added with Yersinia Selective Supplements is intended for the bacteriological processing of clinical specimens such as faeces and rectal swab[2,3] 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. 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.[3]

Incubate aerobically at 28-30°C for 24-48 hours.[3]

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% 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 Irgasan Antimicrobic 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.

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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, the end user can perform its own 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
Y.enterocolitica	ATCC 9610	28-30°C / 18-24H / A	good growth, colonies with red centre
P.aeruginosa	ATCC 27853	28-30°C / 44-48H / A	growth inhibited or partially inhibited, colourless colonies
E.coli	ATCC 25922	28-30°C / 44-48H / A	inhibited
E.faecalis	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 Yersinia Selective Supplement added to dehydrated CIN Agar Base, is tested for productivity and selectivity by comparing the results with previously approved Reference Batch.
Productivity is tested by a quantitative test with the target strains *Y.enterocolitica* ATCC 23715 and; 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. Productivity is also tested by ecometric method with *Y.enterocolitica* DSM 13030 and *Y.enterocolitica* ATCC 9610.
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 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.

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.[6]
- *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.[6]
- The majority of *Y.pseudotuberculosis* strains are inhibited by the concentration of 15 mg/L of cefsulodin.[7]
- Some strains of *Y.enterocolitica* serovar O3 fail to grow on CIN Agar.[7]
- 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 supplement is a qualitative *in vitro* diagnostic, for professional use only; it must be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Antibiotics containing supplements must be handled with suitable protection; consult the Safety Data Sheet before use.
- The supplement and the medium base shall be used in association according to the directions described above.
- Apply Good Manufacturing Practice in the preparation process of plated media.
- The supplement is sterilized by membrane filtration.
- Be careful when opening the metal ring to avoid injury.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplements or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused supplements and the sterilized media inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the supplement 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 of the product are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- 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.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store the product in the original package at 2-8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of plated media and the validation of their shelf life, according to the applied storage conditions (temperature and packaging).

16 - REFERENCES

1. Schiemann D A. Synthesis of a selective agar medium for Yersinia enterocolitica. Can J Microbiol 1979; 25(11):1298-1304,
2. 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
3. Public Health England. Investigations of Faecal Specimens for Enteric Pathogens. UK Standards for Microbiology Investigations. 2014. B 30 Issue 8.1.
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
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
7. Fukushima H. Gomyoda M. Growth of Yersinia pseudotuberculosis and Yersinia enterocolitica Biotype 3B Serotype 03 Inhibited on Cefsulodin-Irgasan-Novobiocin Agar. J Clin Microbiol 1986, 24:116-120

4240011 YERSINIA SELECTIVE SUPPLEMENT

SDS
Regulation (EU) 2020/878

Mixture with hazardous ingredients: cefsulodin sodium and novobiocin

Classification

Eye irritation, category 2	H319	Causes serious eye irritation.
Skin irritation, category 2	H315	Causes skin irritation.
Respiratory sensitization, category 1	H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Skin sensitization, category 1	H317	May cause an allergic skin reaction.

Labelling

Pictogram

Signal word Warning

Hazard statements:

H319	Causes serious eye irritation.
H315	Causes skin irritation.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H317	May cause an allergic skin reaction.

Precautionary statements:

P261	Avoid breathing dust / fume / gas / mist / vapours / spray.
P280	Wear protective gloves / eye protection / face protection.
P342+P311	If experiencing respiratory symptoms: Call a POISON CENTER / doctor / . . .
P304+P340	IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P337+P313	If eye irritation persists: Get medical advice / attention.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	This side up	
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/01
Revision 2	Removal of obsolete classification	2023/04

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