



YERSINIA ITC BROTH BASE POTASSIUM CHLORATE SUPPLEMENT TICARCILLIN IRGASAN ANTIMICROBIC SUPPLEMENT YERSINIA ITC BROTH

Dehydrated culture medium, selective supplements and ready-to-use flasks

1 - INTENDED USE

Liquid enrichment medium for the detection of *Yersinia enterocolitica* in samples of the food chain.

2 – COMPOSITIONS*

YERSINIA ITC BROTH BASE

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	10.00 g
Yeast extract	1.00 g
Magnesium chloride anhydrous	28.10 g [^]
Sodium chloride	5.00 g
Malachite green	0.01 g

TICARCILLIN IRGASAN ANTIMICROBIC SUPPLEMENT (VIAL CONTENTS FOR 250 mL OF MEDIUM)

Ticarcillin	0.25 mg
Irgasan	0.25 mg

POTASSIUM CHLORATE SUPPLEMENT (VIAL CONTENTS FOR 250 mL OF MEDIUM)

Potassium chlorate 5% solution	5 mL
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YERSINIA ITC BROTH (READY-TO-USE FLASKS)

Tryptone	10.00 g
Yeast extract	1.00 g
Magnesium chloride anhydrous	28.10 g [^]
Sodium chloride	5.00 g
Malachite green	0.01 g
Potassium chlorate	1.00 g
Ticarcillin	1.00 mg
Irgasan	1.00 mg
Purified water	1000 mL

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

[^]Equivalent to magnesium chloride hexahydrate 60 g/L.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The genus *Yersinia* comprises Gram-negative coccobacilli, of which three species, *Yersinia pestis*, *Yersinia pseudotuberculosis*, and certain strains of *Yersinia enterocolitica* are of pathogenic importance for humans. *Y. enterocolitica* is ubiquitous, being isolated frequently from soil, water, animals, and a variety of foods.¹ The most common form of disease due to *Y. enterocolitica* is gastroenteritis associated with consumption of contaminated food or water.²

The detection of *Y. enterocolitica* can involve up to four successive steps: first enrichment, second enrichment, plating out, confirmation/identification.

Irgasan Ticarcillin Chlorate (ITC) broth is a selective enrichment liquid medium derived from the modified Rappaport base, developed by Wauters *et al.*³ It was found to be the most efficient method for the recovery of *Y. enterocolitica* biotype 4, serotype 0:3, which is the most common clinical serotype in Europe.^{3,4}

ITC broth is included in ISO 10273⁵ procedure for the detection of *Y. enterocolitica* in samples of the food chain.

The detection method of pathogenic *Y. enterocolitica* recommended by ISO 10273⁵ involves the homogenisation of the sample into PSB broth followed by: 1) direct inoculation onto CIN agar plates, 2) incubation of PSB broth, 3) second enrichment step in ITC broth, 3) alkaline treatment, 4) plating out of the treated enrichment broths onto CIN Agar.

Yersinia ITC Broth Base includes tryptone, providing nitrogen, amino acids and trace elements for microbial growth, yeast extract, a source of vitamins, particularly of the B-group, sodium chloride which contributes to maintaining the osmotic balance of the medium, and the basic selective compounds malachite green and magnesium chloride. Selectivity is improved by the antimicrobials included into selective supplements: irgasan with inhibitory properties against Gram negative bacteria, ticarcillin with bactericide activity mainly on Gram-negative but also on Gram-positive bacteria and potassium chlorate, inhibitory for *Enterobacteriaceae* possessing type A nitrate.⁴

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 11 g in 250 mL of cold purified water. Heat to dissolve completely and sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 47-50 °C and aseptically add the contents of one vial of Potassium Chlorate Supplement (REF 4240065) and one vial of Ticarcillin Irgasan Antimicrobial Supplement (REF 4240070) reconstituted with 2 mL of sterile purified water. Mix well and dispense the medium aseptically in 90 mL amounts into flasks of suitable capacity, so as to obtain the minimum area/volume ratio (relative anaerobiosis).

5 - PHYSICAL CHARACTERISTICS

Yersinia ITC Broth Base

Dehydrated medium appearance	beige-green, fine, homogeneous, free-flowing powder
Solution appearance	blue-green, limpid



**Ticarcillin Irgasan Antimicrobial Supplement**

Freeze-dried supplement appearance short, fragile, white pellet
Reconstituted supplement appearance colourless, limpid

Potassium Chlorate Supplement

Solution appearance colourless, limpid

Final pH of complete medium at 20-25 °C 6.9 ± 0.2

6 - MATERIALS PROVIDED-PACKAGING

Product	Type	REF	Pack
Yersinia ITC Broth Base	Dehydrated medium	4022652	500 g (11.3 L)
Ticarcillin Irgasan Antimicrobial Supplement	Freeze-dried supplement	4240060	10 vials, each for 250 mL of medium
Potassium Chlorate Supplement	Liquid supplement	4240065	10 vials, each for 250 mL of medium
Yersinia ITC Broth	Ready to use flasks	5122652	6 x 90 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, sterile loops, spreaders and pipettes, incubator and laboratory equipment as required, flasks, ancillary culture media and reagents.

8 – SPECIMENS

Products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling. Refer to applicable International Standards and regulations⁵ for the collection, transport, storage, preparation of samples and operate in accordance with good laboratory practice.

9 - TEST PROCEDURE**Detection of *Y. enterocolitica* according to ISO 10273³**

1. Initial suspension: homogenize 25 g of sample into 225 mL of PSB Broth (REF 402270).
2. Transfer 10 mL of PSB suspension into 90 mL of ITC broth and mix.
3. Using the initial PSB suspension, divide a total volume of 1 mL onto two to four CIN agar plates⁴ and spread it over the plates.
4. Invert the CIN plates and incubate at 30 °C for 24 h ± 2 h.
5. Incubate the initial suspension in PSB broth and ITC broth at 25 ° for 44 h ± 4 h.
6. Perform the alkaline treatment by transferring 0.5 mL of the incubated PSB and ITC broths into 4.5 mL of KOH 0.5% in saline solution and by mixing.
7. After 20 ± 5 seconds of the addition of the PSB/ITC enrichments to the KOH solution, streak by means of a loop, the surface of a CIN agar plate and the surface of a chromogenic agar plate⁵ to obtain well-separated colonies.
8. Incubate CIN agar plates at 30 °C for 24 h ± 2 h. Incubate the chromogenic plates according to the instructions for use.
9. Perform the confirmation and bio typing tests according to the methods described by the ISO Standard.

Notes

⁴ CIN Agar Base (REF 401302) + Yersinia Selective Supplement (REF 4240011)

⁵ Chromogenic Yersinia Agar Base (REF 408050) + Chromogenic Yersinia Supplement (REF 4240095).

10 - READING AND INTERPRETATION

Bacterial growth in ITC Broth is evidenced by the development of turbidity.

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 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.

CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
<i>Y. enterocolitica</i> ATCC 23715 + <i>P. aeruginosa</i> ATCC 27853+ <i>P. mirabilis</i> ATCC 29906	25°C/ 44h/ A	> 10 typical colonies after subculture onto CIN Agar

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

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale representative sample of all lots of dehydrated Yersinia ITC Broth Base supplemented with Potassium Chlorate and Ticarcillin Irgasan supplements and ready to use flasks are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

Productivity and selectivity are tested together with the following mixtures of appropriate dilutions of target strains (≤ 100 CFU/tube) and non-target strains (≥ 10000 CFU/tube): *Y. enterocolitica* ATCC 23715+*P. aeruginosa* ATCC 27853+*E. coli* ATCC 8739 and *Y. enterocolitica* DSM 13030+*P. aeruginosa* ATCC 27853+*E. coli* ATCC 8739. After incubation of inoculated tubes at 25°C for 44 hours and sub-culture on CIN Agar the target strains will exhibit a predominant growth on plated medium (> 10 typical colonies).

Moreover, productivity is assessed by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 25°C for 44 hours and recording the highest dilution showing growth in Reference Batch (G_{RB}) and in Test Batch (G_{TB}). Productivity is tested with the following target strains: *Y. enterocolitica* ATCC 23715 and *Y. enterocolitica* ATCC 9610. The productivity index $G_{RB}-G_{TB}$ for each test strain shall be ≤ 1 .

Selectivity is tested by dilution to extinction method with the non-target strain *P. aeruginosa* ATCC 27853 and *P. mirabilis* ATCC 29906. The growth of non-target strains is totally inhibited on the subculture on TSA plates.

13 – LIMITATIONS OF THE METHOD

- The recovery and identification of pathogenic *Yersinia* may be influenced by the type of samples, the enrichment and plating media, level and type of background microflora, the level of pathogenic and non-pathogenic *Yersinia*, serotype of pathogenic *Yersinia* present in foods, and loss of virulence genes during incubation.⁶





- ITC broth performs well for the pathogenic *Y. enterocolitica* serotype O:3 but is less appropriate for other serotypes.⁴
- In foodborne outbreaks investigation, the cold enrichment procedure could be necessary to supplement the general procedure.⁵

14 - PRECAUTIONS AND WARNINGS

- The medium base, the supplements and the ready-to-use flasks are for microbiological control and 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 supplements shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media must be handled with suitable protection. Ticarcillin Irgan Antimicrobial Supplement is classified as hazardous. 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.
- Be careful when opening screw cap flasks and the metal ring of supplements to prevent injury due to breakage of glass.
- Ready-to-use flasks are subject to terminal sterilization by autoclaving followed by aseptically addition of supplements.
- The supplements are sterilized by membrane filtration.
- Each flask of this culture medium is for single use only.
- 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 medium and supplements and the sterilized medium inoculated 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 Sheets 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.

15 - STORAGE CONDITIONS AND SHELF LIFE

Ready-to-use medium in flasks

Upon receipt, store flasks in their original pack at +2°C /+8°C away from direct light. If properly stored, the flasks may be used up to the expiration date. Do not use the flasks beyond this date. Flasks from opened secondary packages can be used up to the expiration date. Opened flasks must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use flasks with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

Dehydrated medium

Upon receipt, store at +10°C /+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 are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

Freeze-dried supplement

Upon receipt, store the product in the original package at +2°C /+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 lyophilized 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).

Liquid supplement

Upon receipt, store the product in the original package at +2°C /+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, the solution should be used immediately. Before use, examine the solution 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 prepared media and the validation of their shelf life, according to the type and the applied storage conditions (temperature and packaging). According to Curtis *et al.* the self-prepared complete medium in flasks may be stored at least 1 month at 2-8°C in screw-capped containers.⁴













16- REFERENCES

1. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 8: *Yersinia enterocolitica*. Rev 10/2017
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. Wauters G, Goossens V, Janssens M, Vandepitte J. New enrichment method for isolation of pathogenic *Yersinia enterocolitica* serogroup O:3 from pork. *Appl Environ Microbiol*. 1988; 54: 851-854.
4. Curtis GDW, Baird RM. Pharmacopoeia of Culture Media for Food Microbiology: Additional Monographs (II). Proceedings of the 6th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Heidelberg 30 March-3 April, 1992. *Int J Food Microbiol* 1993; 17:260-1.
5. ISO 10273:2017 Microbiology of the food chain-Horizontal method for the detection of pathogenic *Yersinia enterocolitica*.
6. American Public Health Association. Compendium of Methods for the Microbiological Examination of Foods, 5th ed. 2015. APHA, Washington, DC.





TABLE OF APPLICABLE SYMBOLS

 or REF Catalogue number	 LOT Batch code	 Manufacturer	 This side up	 Store in a dry place	 Fragile
 Temperature limitation	 Content sufficient for <n> tests	 Consult Instructions for Use	 Use by	 Keep away from direct light	 For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/09

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

