YERSINIA PSB BROTH

Dehydrated culture medium

1 - INTENDED USE

Liquid enrichment medium for the detection of *Yersinia enterocolitica* in food, water, environmental samples.

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

Peptone	5.00 g
Sorbitol	10.00 g
Sodium chloride	5.00 g
Disodium hydrogen phosphate	8.23 g
Sodium dihydrogen phosphate	1.20 g
Bile salts n° 3	1.50 g

*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* 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 stages: first enrichment, second enrichment, plating out, identification. Peptone Sorbitol Bile (PSB) Broth is a selective enrichment liquid medium included in ISO 10273[3], FDA-BAM[1] and APHA[4] procedures for the detection of *Y. enterocolitica* in food, water, and environmental samples.

The detection method of pathogenic *Y. enterocolitica* recommended by ISO 10273[3] 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 4) alkaline treatment, 5) plating out of the treated enrichment broths onto CIN Agar. The detection method recommended by FDA-BAM[1] and APHA[4] involves the enrichment in PSB broth followed by plating onto selective media (MacConkey Agar and CIN Agar plates).

Yersinia PSB Broth includes a peptone providing nitrogen, amino acids and trace elements for microbial growth. Sorbitol is a fermentable carbohydrate, source of carbon and energy. Bile salts n° 3 limits the growth of Gram-positive bacteria. Sodium chloride contributes to maintaining the osmotic balance of the medium. Phosphates are used as buffering agents to control the pH in the medium.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 31 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Dispense into tubes or flasks of suitable capacity to obtain portions appropriate for the test samples. Sterilise by autoclaving at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared tubes appearance	pale yellow, limpid
Final pH at 20-25 °C	7.6 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Yersinia PSB Broth	Dehydrated medium	4022702	500 g (16 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 – SPECIMENS

Food, water, environmental samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[1,3,4]

9 - TEST PROCEDURE

Detection of *Y. enterocolitica* according to ISO 10273[3]

1. Initial suspension: homogenize 25 g of sample into 225 mL of Yersinia PSB Broth.
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 plates^ 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 the ITC broth flasks at 25 °C 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 plate§ 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 biotyping tests according to the methods described by the ISO Standard.

Notes

* Yersinia ITC Broth Base (REF 402265) + Potassium Chlorate Supplement (REF 4240065) + Ticarcillin Irgasan Antimicrobic Supplement (REF 4240060).

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

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

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10 - READING AND INTERPRETATION

Bacterial growth in Yersinia PSB 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
Y. enterocolitica ATCC 23715 + *P. aeruginosa* ATCC 27853	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 a representative sample of all lots of dehydrated Yersinia PSB Broth is 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 stains (≤ 100 CFU/tube) and non-target strains (≥ 1000 CFU/tube): *Y. enterocolitica* ATCC 23715+*P. aeruginosa* ATCC 27853 and *Y. enterocolitica* ATCC 9610+*P. aeruginosa* ATCC 27853. After incubation of inoculated tubes at 25°C for 44 hours and sub-culture on CIN Agar the target strains will show 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 (Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following target strains: *Y. enterocolitica* ATCC 23715 and *Y.enterocolitica* ATCC 9610. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.
Selectivity is tested by dilution to extinction method with the non-target strain *E. faecalis* ATCC 29212. After incubation the growth of *E. faecalis* is partially inhibited.

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.[4]
- In foodborne outbreaks investigation, the cold enrichment procedure could be necessary to supplement the general procedure.[3]

14 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- 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 this 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.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- 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.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material 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.
- 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 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).
The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the shelf life of the finished products, according to the type and the storage method (temperature and packaging).

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. ISO 10273:2017 Microbiology of the food chain-Horizontal method for the detection of pathogenic Yersinia enterocolitica.
4. American Public Health Association. Compendium of Methods for the Microbiological Examination of Foods, 5th ed. 2015. APHA, Washington, DC.

TABLE OF APPLICABLE SYMBOLS

REF Or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

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.

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