



# RAPPAPORT VASSILIADIS ENRICHMENT SALMONELLA BROTH EP

Dehydrated culture medium

## 1 - INTENDED USE

Liquid medium for the selective enrichment of *Salmonella* in non-sterile pharmaceutical products.

## 2- COMPOSITION - TYPICAL FORMULA <sup>^\*</sup>

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Soy peptone	4.500 g
Sodium chloride	8.000 g
Potassium dihydrogen phosphate	0.600 g
Dipotassium hydrogen phosphate	0.400 g
Magnesium chloride anhydrous	13.580 g <sup>^</sup>
Malachite green oxalate	0.036 g

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

<sup>^</sup> corresponding to magnesium chloride hexahydrate 29.0 g/L

## 3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Rappaport in 1956<sup>1</sup> devised an enrichment broth for *Salmonella* that included malachite green and magnesium chloride as inhibitors. Vassiliadis in 1976<sup>2</sup> modified the Rappaport medium by reducing to one-third the concentration of malachite green and incubating at 43°C instead of 37°C. Van Schothorst and Renaud<sup>3</sup> reported that the use of soy peptone instead of animal peptone improved recovery rates of *Salmonella*. Rappaport Vassiliadis Enrichment Salmonella Broth EP is based on the formulation of Van Schothorst<sup>3</sup> and Renaud and meets the requirements of European Pharmacopoeia.<sup>4</sup>

The medium is used as a selective enrichment medium for the isolation of *Salmonella* from non-sterile pharmaceutical products with after the pre-enrichment in Tryptic Soy Broth, according to the method reported by European Pharmacopoeia.<sup>4</sup>

Rappaport Vassiliadis Enrichment Salmonella Broth EP differs from the ISO standard medium (REF 401781) by slightly different concentrations of sodium chloride and phosphate buffer.

The efficiency of this enrichment medium is based on the ability of *Salmonella* spp. to multiply at relatively high osmotic pressures, at relatively low pH values, at a high temperature and with reduced nutritional requirements.<sup>5</sup>

Essential growth factors are provided by soy peptone; malachite green is inhibitory to organisms other than salmonellae; the high osmotic pressure of the medium due to the high concentrations of magnesium chloride, together with the acid pH, act as inhibitors of the saprophytic flora, favouring the growth of *Salmonella* in the broth. Magnesium chloride in addition counteracts the toxic effect of malachite green for salmonellae. Phosphates are used as buffering agents to control the pH in the medium.

## 4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 27.1 g in 1000 mL of cold purified water. Heat gently to dissolve, distribute 10 mL into screw-cap tubes and sterilise by autoclaving at 115°C for 15 minutes.

## 5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	blue-green, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	blue, limpid
Final pH at 20-25 °C	5.2 ± 0.2

## 6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Rappaport Vassiliadis Enrichment Salmonella Broth EP	Dehydrated medium	4019792	500 g (18.4 L)

## 7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, incubator and laboratory equipment as required, Erlenmeyer flasks, tubes, bottles, sterile loops and pipettes, ancillary culture media and reagents.

## 8 - SPECIMENS

Non-sterile pharmaceutical products. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to European Pharmacopoeia.<sup>4</sup>

## 9 - TEST PROCEDURE

The following method is a summary of the technique recommended by European Pharmacopoeia.<sup>4</sup>

- Inoculate not less than 10 g or 10 mL into 90 mL of Tryptic Soy Broth (REF 402155), mix and incubate at 30-35°C for 18-24 hours.
- Transfer 0.1 mL of the culture to 10 mL of Rappaport Vassiliadis Salmonella Enrichment Broth EP and incubate at 30-35 °C for 18-24 h.
- Subculture on plates of XLD agar (REF 402208) and incubate at 30-35 °C for 18-48 h.

## 10 - READING AND INTERPRETATION

After incubation, the growth of organisms in the enrichment broth is indicated by a milky appearance of the broth or by turbidity.

The possible presence of *Salmonella* is indicated by the growth of well-developed, red colonies, with or without black centres on XLD Agar plates. This is confirmed by identification tests.

## 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
S. Typhimurium ATCC 14028	30-35 °C/ 18-24h/ A	good growth
S. aureus ATCC 25923	30-35 °C/ 18-24h/ 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 Rappaport Vassiliadis Enrichment Salmonella Broth EP Broth is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

Productivity is tested by dilution to extinction method, by inoculating less than 100 CFU/tube of the target organism S. Typhimurium ATCC 14208 and incubating at 30-35 °C for 18-24 hour. The target strain exhibits a good growth.

Selectivity is evaluated by inoculating more than 100 CFU/tube of the non-target strain S. aureus ATCC 25923 and incubating at 30-35 °C for 18-24 hour. The growth of the non-target strain is totally inhibited.

### 13 - LIMITATIONS OF THE METHOD

- RVS Broth is inhibitory for S.Typhi.
- After the selective enrichment, 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.

### 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.
- 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 medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium 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 products are available on the website [www.biolifeitaliana.it](http://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 (tubes/bottles) and the storage method (temperature and packaging). According to ISO 6579 prepared tubes and flasks of RVS Broth may be stored at 2-8°C for up to three months.<sup>6</sup>

### 16 - REFERENCES

- Konforti K, Navon B, Rappaport F. A new enrichment medium for certain salmonellae. J Clin Pathol 1956; 9:261-266.
- Vassiliadis P, Pateraki E, Papiconomou N, Papadakis J, Trichopoulos D. Nouveau procédé d'enrichissement de salmonella. Ann. Microb. Irist, Pasteur 1976; 127 B: 195.
- Van Schothorst M, Renaud AM. Dynamics of salmonella isolation with modified Rappaport's medium (R10). J Appl Bacteriol 1983; 54:209
- European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.13 Microbiological Examination of non-sterile products: test for specified micro-organisms: 01/2021:20631.
- Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:254-255.
- ISO 6579:2017. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella —Part 1: Detection of Salmonella spp

### TABLE OF APPLICABLE SYMBOLS

 or  Catalogue number	 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 2	Updated layout and content	2022/08

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



**Biolife**

## Instructions for use

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