RAPPAPORT VASSILIADIS SOY (RVS) BROTH

Dehydrated and ready-to-use culture medium

RVS Broth. On the left: uninoculated medium; on the right medium after the growth of Salmonella spp.

1 - INTENDED USE

Liquid medium for the selective enrichment of *Salmonella* in food, water and environmental samples.

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

Soy peptone	4.500 g
Sodium chloride	7.200 g
Potassium dihydrogen phosphate	1.260 g
Dipotassium hydrogen phosphate	0.180 g
Magnesium chloride anhydrous	13.400 g
Malachite green oxalate	0.036 g

^ The reported formulation uses anhydrous ingredients to better preserve the powder. The Biolife formulation per litre corresponds to the ISO Standard formulation, which refers to a total volume of 1110 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

Rappaport in 1956[1] devised an enrichment broth for *Salmonella* that included malachite green and magnesium chloride as inhibitors. Vassiliadis in 1976[2] 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[3] reported that the use of soy peptone instead of animal peptone improved recovery rates of *Salmonella*. Rappaport Vassiliadis Soy (RVS) Broth is based on the formulation of Van Schothorst and Renaud and meets the requirements of ISO 6579-1[4] and ISO 19250[5].
Rappaport Vassiliadis Soy (RVS) Broth is used as a selective enrichment medium for the isolation of *Salmonella* from food, water and environmental specimens with incubation at 41.5 °C, after the pre-enrichment in Buffered Peptone Water.
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.[6]
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*. 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 26.6 g in 1000 mL of cold purified water. Heat 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, clear
Final pH at 20-25 °C	5.2 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Rappaport Vassiliadis Soy (RVS) Broth	Dehydrated medium	4019812	500 g (18.7 L)
RVS Broth	Ready-to-use tubes	551981	20 x 10 mL
RVS Broth	Ready-to-use flasks	5119812	6 x 100 mL

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

Food, water and environmental specimens. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.[4,5]

9 - TEST PROCEDURE

Food samples

The following method is a summary taken from the ISO 6579-1.[4]

1. Prepare the test sample in accordance with the specific International Standard dealing with the product concerned. In general, an amount of test portion is added to a quantity of pre-warmed Buffered Peptone Water (REF 401278) to yield a tenfold dilution (e.g., 25 g test portion is mixed with 225 mL of Buffered Peptone Water).
2. Incubate between 34 °C and 38 °C for 18 h ± 2 h.
3. Transfer 0.1 mL of the culture obtained in Buffered Peptone Water to a tube containing 10 mL of the RVS broth or to the surface of a MSRV Agar plate (REF 401982).

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4. Transfer 1 mL of the culture obtained in Buffered Peptone Water to a tube containing 10 mL of Muller Kauffmann Tetrathionate Novobiocin Broth (REF 401745 - MKTTn Broth).
5. Incubate the inoculated RVS Broth (or MSRV plates) at 41.5 °C ± 1 for 24 h ± 3 h.
6. Incubate the inoculated MKTTn Broth between 34 °C and 38 °C for 24 h ± 3 h.
7. From RVS Broth or MSRV medium and MKTT Broth transfer a loopful of growth on a plate of XLD Agar (code 402208) and on another selective medium for *Salmonella* based on different diagnostic characteristics to those of XLD agar (e.g. Chromogenic Salmonella Agar REF 405350). With MSRV medium positive plates use a 1 µL loop, with MKTTn Broth use a 10 µL loop.
8. Incubate the XLD plates inverted between 34 °C and 38 °C and examined after 24 h. Incubate the second selective plating-out medium in accordance with the instructions for use.

NOTES

After incubation, it is permissible to store the pre-enriched sample and selective enrichment at 2-8 °C for a maximum of 72 h.[4]
In dried milk products and cheese, *Salmonella* may be sub lethally injured. Incubate the selective enrichment media from these products for an additional 24 h ± 3 h. When investigating outbreak samples, this additional incubation time may also be beneficial.[4]
Refer to the ISO Standard for the detailed procedures.

Water samples

The following method is a summary taken from the ISO 19250.[5]

1. Non-selective pre-enrichment for volumes < 10 mL: inoculate 50 mL of Buffered Peptone Water with the sample or dilutions thereof and incubate at 36 ± 2 °C for 18 ± 2 h.
2. Non-selective pre-enrichment for volumes > 10 mL: filter a volume of water appropriate for the water being examined. Place the membrane filter into 50 mL of Buffered Peptone Water. Alternatively, add the sample to the same volume of double strength Buffered Peptone Water. Note that this latter procedure is not suitable for mineral waters with high salt content or sea water.
3. Incubate the cultures at 36 ± 2 °C for 18 ± 2 h.
4. Transfer 0.1 mL to 10 mL of RVS Broth and incubate at 41.5 °C ± 1 °C for 24 h ± 3 h and, if necessary for 48 ± 4 h.
5. From the tubes of RVS Broth transfer a loopful of growth on a plate of XLD Agar ISO Formulation (code 402208) and on another selective medium for *Salmonella* based on different diagnostic characteristics to those of XLD agar (e.g., Chromogenic Salmonella Agar REF 405350).
6. Incubate the XLD plates inverted between 34 °C and 38 °C and examined after 24 h. Incubate the second selective plating-out medium in accordance with the instructions for use.

NOTES

For waste water it has been shown that shorter incubation times of pre-enrichment culture or direct inoculation of the sample in selective medium produces better results.[5]
To detect slow growing *Salmonella* species, incubate the RVS broth for a total of 48 ± 4 hours.[5]
Refer to the ISO Standard for the detailed procedures.

10 - READING AND INTERPRETATION

After incubation, the growth of organisms in RVS Broth is indicated by a milky appearance of the broth or by turbidity.
Refer to the instructions for use of the two plated media for the description of *Salmonella* colony characteristics.
Mark suspect colonies on each plate. Select suspect colony for subculture and confirmation.
Biochemical confirmation tests include: TSI Agar, Urea Agar, L-Lysine Decarboxylase Medium, detection of β-galactosidase (optional), indole detection (optional).[4] Serological confirmation includes the detection of the presence of *Salmonella* O- and H-antigens.
Biochemical confirmation can be substituted with the rapid MUCAP Test (REF 191500). All the colonies MUCAP Test positive must be serologically confirmed.

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 + *P. aeruginosa* ATCC 27853 + *E. coli* ATCC 25922	41.5 °C ± 1 °C / 24 h ± 3/ A	> 10 typical colonies after subculture on XLD Agar
E. faecalis ATCC 29212	41.5 °C ± 1 °C / 24 h ± 3/ A	< 100 colonies after subculture on TSA
E. coli ATCC 25922	41.5 °C ± 1 °C / 24 h ± 3/ A	partially inhibited after subculture on TSA

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

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale representative samples of all lots of dehydrated and ready-to-use Rappaport Vassiliadis Soy (RVS) Broth are 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 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 41.5 ± 1°C for 24 hours, sub-culturing on Tryptic Soy Agar plates 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: S. Typhimurium ATCC 14208, S. Enteritidis ATCC 13076, The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.
Productivity and selectivity are tested together with mixtures of approximately 100 CFU of target organisms and 1000 CFU of non- target organisms per test tubes, incubating 41.5 ± 1°C for 24 hours. Mixture of target and non-target strains: S. Typhimurium ATCC 14028 +*E. coli* ATCC 25922+ *P. aeruginosa* ATCC 27853. After incubation of inoculated tubes and sub-culture on XLD Agar plates, the target strain will show more than 10 colonies per plate
Moreover, selectivity is evaluated by inoculating approximately 10,000 CFU/tube of non-target organisms and incubating at 41.5 ± 1°C for 24 hours and sub-culturing on Tryptic Soy Agar plates. Selectivity is tested with the following non-target strains: *E. coli* ATCC 25922, *E. faecalis* ATCC 2921. *E. coli* is partially inhibited while CFU of *E. faecalis* shall be less than 10 on the sub-cultured plates of Tryptic Soy Agar.

13 - LIMITATIONS OF THE METHOD

- The combination of malachite green, magnesium chloride and low pH does not allow the growth of certain *Salmonella* serovars such as *Salmonella* Typhi and *Salmonella* Paratyphi A.

- Colonies of presumptive *Salmonella* must be sub cultured and their identity confirmed by means of appropriate biochemical and serological tests.

14 - PRECAUTIONS AND WARNINGS

- This culture medium 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.
- Be careful when opening screw cap flasks and tubes to prevent injury due to breakage of glass.
- Ready-to-use flasks and tubes are subject to terminal sterilization by autoclaving.
- Each ready-to-use tubes and 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 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 Sheets of the products 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/tubes

Upon receipt, store flasks/tubes in their original pack at 2-8°C away from direct light. If properly stored, the flasks/tubes may be used up to the expiration date. Do not use the flasks/tubes beyond this date. Flasks/tubes from opened secondary packages can be used up to the expiration date. Opened flasks/tubes must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use flasks/tubes 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).

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 6579-1 prepared tubes and flasks of RVS Broth may be stored at 2-8°C for up to three months.[4]

16 – REFERENCES

1. Konforti K, Navon B, Rappaport F. A new enrichment medium for certain salmonellae. J Clin Pathol 1956; 9:261-266.
2. Vassiliadis P, Pateraki E, Papiconomou N, Papadakis J, Trichopoulos D. Nouveau procède d'enrichissement de salmonella. Ann. Microb. Irist, Pasteur 1976; 127 B: 195.
3. Van Schothorst M, Renaud AM. Dynamics of salmonella isolation with modified Rappaport's medium (R10). J Appl Bacteriol 1983; 54:209
4. ISO 6579-1:2017 Microbiology of the food chain -- Horizontal method for the detection, enumeration and serotyping of Salmonella -- Part 1: Detection of Salmonella spp. - ISO 6579-1:2017/Amd 1:2020 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp. — Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC
5. ISO 19250:2010. Water quality -- Detection of Salmonella spp.
6. 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.

TABLE OF APPLICABLE SYMBOLS

REF 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 2	Updated layout and content	2022/08

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

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