INSTRUCTIONS FOR USE

RAPPAPORT VASSILIADIS (RV) BROTH

Ready-to-use tubes

Rappaport Vassiliadis (RV) Broth
from the left: un-inoculated tube and *S.*Enteritidis growth.

1 - INTENDED USE

In vitro diagnostic device. Liquid medium for the selective enrichment of *Salmonella* from food, environmental and clinical samples.

2 - COMPOSITION -TYPICAL FORMULA *

Tryptone	4.540 g
Potassium dihydrogen phosphate	1.450 g
Sodium chloride	7.200 g
Magnesium chloride anhydrous	13.300 g
Malachite green oxalate	0.036 g
Purified water	1000 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 Vassiliadis (RV) Broth is prepared according to the R25/37 formulation proposed by Rappaport in 1956[1] and modified by Vassiliadis in 1976[2], and called R10/43. Two major modifications were introduced in the composition and use: one modification consisted of the reduction to one-third of the amount of malachite green and the other in the incubation at 43°C instead of 37°C.
From 1977 to 1981, in eleven studies, Rappaport Vassiliadis (RV) Broth has been compared to the standardized Muller Kauffmann Tetrathionate broth (MK broth) recommended as a reference method by the International Standards Organization; in all these studies the RV broth was superior to the MK broth in the isolation of salmonellas from naturally contaminated meat products, sewage and faeces of healthy pigs, after pre-enrichment in buffered peptone water.[3]
Rappaport Vassiliadis (RV) Broth is recommended by the FDA BAM[4] as a selective enrichment broth for the isolation of Salmonella.
The medium is also reported as a selective enrichment broth for Salmonella spp. other than Salmonella Typhi in human stool samples.[5-7]
Tryptone is a source of nitrogen and carbon for microbial growth; malachite green is inhibitory towards coliforms; 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 development of Salmonella in the broth. Magnesium chloride suppresses the toxic effects of malachite green towards Salmonellae; potassium dihydrogen phosphate acts as a buffer system.
An extensive review of the scientific papers published on Rappaport Vassiliadis Broth was published by Vassiliadis in 1983.[3]

4 - PHYSICAL CHARACTERISTICS

Medium appearance	blue, limpid
Final pH at 20-25°C	5.2 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Rappaport Vassiliadis (RV) Broth	Ready-to-use tubes	551980	20 x 10 mL glass tubes, 17x125 mm, flat bottom, aluminium screw-cap. Packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Rappaport Vassiliadis (RV) Broth may be inoculated with human clinical specimens such as faeces or rectal swab. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied. For food and environmental samples refer to the quoted reference.[4]

8 - TEST PROCEDURE

Faeces:
- Inoculate the tube of Rappaport Vassiliadis (RV) Broth with a substantial loop of faeces or with 50 - 100 µL of liquid faeces. Rectal swabs received fresh or in transport medium should be rinsed thoroughly in 1 mL of saline.
- Incubate the inoculated tubes in aerobic atmosphere at 41.5 ± 0.5°C (or at 42.0 ± 0.2°C in a water bath) for 24 ± 2 hours.[8]

Food:
- Inoculate 25 g of sample in 225 ml of Buffered Peptone Water (REF 401278) and incubate at 35-37°C for 16-20 hours.
- Transfer 0.1 mL to 10 mL of Rappaport Vassiliadis (RV) Broth and incubate at 41.5 ± 0.5°C (or at 42.0 ± 0.2°C in a water bath) for 24 ± 2 hours. [4,8]
- Streak a loopful of incubated and mixed RV Broth on XLD Agar, and on a second plating medium.[4]

For a detailed description of methods for detecting *Salmonella* in food, refer to the cited literature.[4]

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9 - READING AND INTERPRETATION

After incubation, the growth of organisms is indicated by a milky appearance of the broth or by turbidity.
Subculture by streaking a loopful of broth on selective enteric plating media. The plating media should be chosen as a combination of greater and lesser inhibitory selective agars.

10 - 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.Enteritidis ATCC 13076	41.5 ± 0.5 °C / 22-26 h / A	good growth after subculture to Tryptic Soy Agar plate
S.Typhimurium ATCC 14028	41.5 ± 0.5 °C / 22-26 h / A	good growth after subculture to Tryptic Soy Agar plate
E.coli ATCC 25922	41.5 ± 0.5 °C / 22-26 h / A	growth partially inhibited after subculture to Tryptic Soy Agar plate

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

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of ready to use tubes of Rappaport Vassiliadis (RV) Broth and of the raw material used for the ready-to-use tube production (dehydrated Rappaport Vassiliadis (RV) Broth REF 401980) 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 ± 0.5°C for 22-26 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 13076, S.Enteritidis ATCC 14028. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.
Selectivity is evaluated with dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of non-target organisms in test tubes, incubating at 41.5 ± 0.5°C for 22-26 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 29212, *S.aureus* ATCC 25223. *E.coli* and *S.aureus* are partially inhibited and the selectivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≥1; *E.faecalis* CFU's shall be less than 10 on the sub-cultured plates of Tryptic Soy Agar.

12 - LIMITATIONS OF THE METHOD

- Rappaport Vassiliadis (RV) Broth is inhibitory for S.Typhi. The medium is therefore not indicated for the diagnosis of typhoid fevers. Isolation techniques should include a variety of enrichment broths and isolation media.
- For the enrichment of human faecal specimens, the most recommended media by microbiological manuals and procedures are sodium selenite containing broths.[5,9]
- After the enrichment in Rappaport Vassiliadis (RV) Broth, 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.
- This culture medium is 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.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- 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 these products do not contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use tubes 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each tube is for single use only; do not transfer or subdivide the tube content in other containers.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.
- Ready-to-use tubes of Rappaport Vassiliadis (RV) Broth are subject to terminal sterilization by autoclaving.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the tubes inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet 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* diagnostics.
- 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.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store tubes in their original pack at 2-8°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. After opening the box, the tubes can be used up to the expiration date. Opened tubes must be used immediately. Before use, check the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g. microbial contamination, atypical colour, precipitate).

15 – REFERENCES

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3. Vassiliadis P. The Rappaport Vassiliadis enrichment Broth for the isolation of salmonellas: an overview. J App Bact 1983; 54: 69
4. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev 07/2020
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7. Bockemühl, J. 1992. *Enterobacteriaceae. In:* Burkhardt, F. (ed.). Mikrobiologische Diagnostik. Thieme Verlag, Stuttgart, New York.
8. Peterz M, Wiberg C, Norberg P. The effect of incubation temperature and magnesium chloride concentration on growth of salmonella in home-made and in commercially available dehydrated Rappaport-Vassiliadis broths. J Appl Bacteriol. 1989 Jun;66(6):523-8.
9. Public Health England- UK Standards for microbiology investigations (UK SMI): SMI B 30: investigation of faecal specimens for enteric pathogens. Issue 8.1, 04/2014.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Do not reuse	Recyclable pack 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
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2021/04
Revision 2	Removal of obsolete classification	2023/04

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

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