INSTRUCTIONS FOR USE

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RAPPAPORT VASSILIADIS (RV) BROTH

Dehydrated culture medium

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

1 - INTENDED USE

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

2- COMPOSITION - TYPICAL FORMULA *

(AFTER RECONSTITUTION WITH 1 L OF WATER)

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

*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, the RV enrichment 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 reported by the FDA BAM[4] as a selective enrichment broth for the isolation of *Salmonella*.
The ISTISAN 96/35[5] Report indicates the following scheme for the selective enrichment of *Salmonella* in food:
Non-regulated food: Rappaport Vassiliadis (RV) (42°C) + Selenite Cystine Broth (37°C)
Milk and derivatives: Muller Kauffmann (42°C) + Selenite Cystine Broth (37° C)
Fresh eggs: Rappaport Vassiliadis (RV) (42°C) + Muller Kauffmann (42°C)
Molluscs: MSRV (42°C)
The medium is also recommended as a selective enrichment broth for *Salmonella* spp. other than *Salmonella* Typhi in human stool samples by Kist et al.[6] and by Burkhardt[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 the Rappaport Vassiliadis Broth was published by Vassiliadis in 1983.[3]

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 26.5 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.

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5 - PHYSICAL CHARACTERISTICS

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

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Rappaport Vassiliadis (RV) Broth	Dehydrated medium	4019802 4019804	500 g (18.8) 5 kg (188 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, incubator and laboratory equipment as required, Erlenmeyer flasks, tubes, bottles, sterile loops and swabs, ancillary culture media and reagents for the identification of the colonies.

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8 - 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 references.[4,5]

9 - 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 42 ± 1°C for 18-24 hours.

Food:
- Inoculate 25 g of sample in 225 ml of Buffered Peptone Water (code 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 42 ± 1°C for 24 hours
- From the tubes of Rappaport Vassiliadis Broth transfer a loopful of growth on a plate of XLD Agar (code 402208) and on another selective medium for *Salmonella*.

For a detailed description of methods for detecting *Salmonella* in food, refer to the cited literature.[4,5]

10 - READING AND INTERPRETATION

After incubation, the growth of organisms is indicated by a milky appearance of the broth or by 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
*S.*Enteritidis ATCC 13076	42 ± 1°C / 18-24h / A	good growth after subculture to Tryptic Soy Agar plate
*S.*Typhimurium ATCC 14028	42 ± 1°C / 18-24h / A	good growth after subculture to Tryptic Soy Agar plate
E.coli ATCC 25922	42 ± 1°C / 18-24h / A	growth partially inhibited after subculture to Tryptic Soy Agar plate

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 (RV) 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 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 42 ± 1°C for 18-24 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.
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 42 ± 1°C for 18-24 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 29212, *S.aureus* ATCC 25923. *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.

13 - 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.
- For the enrichment of human faecal specimens, the most recommended media by microbiological manuals and procedures are selenite containing broths.[8,9]
- 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.

14 - 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.
- 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.

- 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* diagnostic.
- 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).

16 – REFERENCES

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TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/09
Revision 2	Update of "precautions and warnings" and "storage conditions and shelf life"	2022/03
Revision 3	Removal of obsolete classification	2023/04

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

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