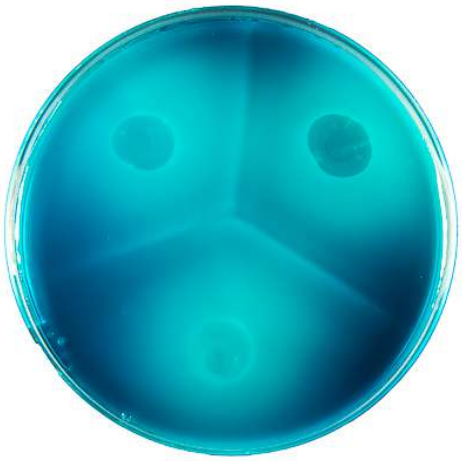
RAPPAPORT VASSILIADIS SEMI-SOLID MEDIUM MODIFIED (MSRV)

NOVOBIOCIN ANTIMICROBIC SUPPLEMENT

Dehydrated culture medium and selective supplement



MSRV medium: migration of *Salmonella* Typhimurium

1 - INTENDED USE

Basal medium and supplement for selective enrichment/isolation of mobile *Salmonella* strains from food and animal feed, animal faeces and environmental samples from primary production stage.

2 – COMPOSITION*

MSRV MEDIUM – DEHYDRATED MEDIUM

TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER

Enzymatic digest of animal and plant tissue	4.6 g
Acid digest of casein	4.6 g
Sodium chloride	7.3 g
Potassium dihydrogen phosphate	1.5 g
Magnesium chloride anhydrous	10.9 g
Agar	2.7 g
Malachite green oxalate	40.0 mg

NOVOBIOCIN ANTIMICROBIC SUPPLEMENT - VIAL CONTENT

Novobiocin	10 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Modified Semisolid Rappaport Vassiliadis (MSRV) medium is a semisolid modification of Rappaport Vassiliadis enrichment broth, originally described by De Smedt *et al*[1] in 1986 with a novobiocin concentration of 20 mg/L. MSRV medium enables the sensitive determination of motile *Salmonella* strains in contaminated food or other samples. The use of this medium following pre-enrichment or selective enrichment gave better results in the isolation of *Salmonella* than conventional methods.[2-4]

Veenman et al.[5] demonstrated the presence of larger migration zones on MSRV medium with a lower concentration of novobiocin and the influence of novobiocin on bacterial motility.

MSRV medium with a novobiocin concentration of 10 mg/L is recommended by ISO 6579-1[6] for the determination of *Salmonella* in food, animal feed samples, environmental samples from the food production area, as an alternative to RVS broth and as the only selective enrichment medium for samples from the primary production stage.

The principle of the method is based on the ability of salmonellae to move from the point of inoculation through the selective medium, more rapidly than other competitive microorganisms, producing opaque halos of growth.

Essential growth factors are provided by peptones. Malachite green, the high osmotic pressure due to the high concentration of magnesium chloride, the presence of novobiocin and the acid pH, act as inhibitors of the saprophytic flora, promoting the growth of motile *Salmonella* strains. 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 31.6 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Do not autoclave. Cool to approximately 47-50°C.

ISO 6579-1 formulation

Add the contents of one vial of Novobiocin Antimicrobic Supplement (REF 4240045), reconstituted with 5 mL of sterile purified water. Mix well and pour 15-20 mL into sterile Petri dishes and leave to dry for one hour. Novobiocin concentration in final medium: 10 mg/L

Original De Smedt formulation

Add the contents of two vials of Novobiocin Antimicrobic Supplement (REF 4240045), reconstituted with 5 mL of sterile purified water. Mix well and pour 15-20 mL into sterile Petri dishes and leave to dry for one hour. Novobiocin concentration in final medium: 20 mg/L

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	blue-green, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	blue, clear, semisolid medium
Freeze-dried selective supplement	short, dense, white pellet; colourless and clear solution after reconstitution
Final pH of complete medium (at 20-25°C)	5.2 ± 0.2

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Rappaport Vassiliadis Semisolid Medium (MSRV)	Dehydrated medium	4019822	500 g (15.8 L)
Novobiocin Antimicrobic Supplement	Freeze-dried supplement	4240045	10 vials, 10 mg/vial

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Water-bath, sterile inoculation loops and needles, sterile pipettes, incubator and laboratory equipment as required, sterile Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents.

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8 – SPECIMENS

Products intended for human consumption and the feeding of animals, environmental samples in the area of food production and food handling, samples from the primary production stage such as animal faeces, dust, and swabs. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to the applicable International Standards.

9 - TEST PROCEDURE

Summary of ISO 6579 procedure for food, animal feed samples, and environmental samples from the food production area.[6]

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 the surface of a MSRV Agar plate or to a tube containing 10 mL of RVS broth (REF 401981). Inoculate the MSRV agar with one to three equally spaced spots on the surface of the medium.
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 MSRV Agar plates (or RVS broth tubes) at 41.5 °C ± 1 for 24 h ± 3 h. Do not invert the plates.
6. Incubate the inoculated MKTTn Broth between 34 °C and 38 °C for 24 h ± 3 h.
7. From MSRV medium (or RVS broth) and MKTTn 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). With MSRV medium positive plates use a 1 µL loop, with MKTT 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.

Summary of ISO 6579 procedure for samples from the primary production stage.[6]

After pre-enrichment in Buffered Peptone Water inoculate only the MRSV medium as described above. However, the sensitivity of the method can be improved by using a second selective enrichment procedure, e.g. MKTTn broth incubated at 41.5 °C for 24 h.

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.[6]
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.[6]
Refer to the ISO Standard for the detailed procedures.

10 - READING AND INTERPRETATION

Suspect MSRV plates will show a grey-white, turbid zone extending out from the inoculated drop. If the plates are negative after 24 hours, re-incubate for a further 24 h ± 3 h.
Biochemical confirmation tests include: TSI Agar, Urea Agar, L-Lysine Decarboxylase Medium, detection of β-galactosidase (optional), indole detection (optional).[6] 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.[6]

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
S. Enteritidis ATCC 13076	41.5 °C ± 1 °C / 24-48 h / A	growth with turbid zone
S. Typhimurium ATCC 14028	41.5 °C ± 1 °C / 24-48 h / A	growth with turbid zone
E. faecalis ATCC 29212	41.5 °C ± 1 °C / 48 h / A	inhibited
E. coli ATCC 25922	41.5 °C ± 1 °C / 48 h / A	inhibited

12- PERFORMANCES CHARACTERISTICS

Prior to release for sale, representative samples of all lots of dehydrated Rappaport Vassiliadis Semisolid Medium (MSRV) supplemented with Novobiocin Antimicrobic Supplement (10 mg/L), are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
The productivity characteristics are assessed by inoculating 0.1 mL of target strains suspensions on the surface of MSRV medium plate, at least 5 mm from the edge. Tested target strains: S. Typhimurium ATCC 14028, S. Enteritidis ATCC 13076. The diameters of migration zones are measured after incubation at 41.5 °C ± 1 °C for 24 and 48 hours. If the zones diameters in the Test Batch are comparable with the measurements obtained in the Reference Batch, the Test Batch is considered compliant.
The selectivity is assessed with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of *E. coli* ATCC 25922, and *E. faecalis* ATCC 29212. After 48 hours of incubation at 41.5 °C, the growth of *E. coli* is totally or partially inhibited and no migration zones are observed, while the growth of *E. faecalis* is totally inhibited.

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.
- The medium is not suitable for detecting non-motile strains of *Salmonella*, which occur in any case with a very low incidence (less than 0.1%). If the presence of non-motile strains is suspected, a conventional method with pre-enrichment and selective enrichment is recommended.
- Colonies of presumptive *Salmonella* must be sub cultured and their identity confirmed by means of appropriate biochemical and serological tests.

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplement are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Novobiocin Antimicrobic Supplement is classified as hazardous. Before use, consult the Safety Data Sheets.
- 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 the 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.
- Be careful when opening the metal ring of the supplement vials to avoid injury.
- The supplement is sterilized by membrane filtration.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets 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

Dehydrated medium

Upon receipt, store at +10°C /+30°C away from direct light. The medium is very hygroscopic: keep the bottle lid very tight in an air-dry room. 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).

Freeze-dried supplement

Upon receipt, store the product in the original package at 2-8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes/bottles) and the applied storage conditions (temperature and packaging). According to ISO 6579-1 the self-prepared plates can be stored with surface upwards, and protected from drying for up to two weeks at +2°C +8°C in the dark.[6]

16 - REFERENCES

1. De Smedt JM, F Bolderdijk RF, Rappold H, Lautenschlaeger D. Rapid Salmonella Detection in Foods by Motility Enrichment on a Modified Semi-Solid Rappaport-Vassiliadis Medium. J Food Prot. 1986 Jul;49(7):510-514.
2. De Smedt JM, Chartron S, Cordier JL, Graff E, Hoekstra H, Lecoupeau JP, Lindblom M, Milas J, Morgan RM, Nowacki R, et al. Collaborative study of the International Office of Cocoa, Chocolate and Sugar Confectionery on Salmonella detection from cocoa and chocolate processing environmental samples. Int J Food Microbiol. 1991 Aug;13(4):301-8.
3. De Smedt JM, Bolderdijk R, Milas J. Salmonella detection in cocoa and chocolate by motility enrichment on modified semi-solid Rappaport-Vassiliadis medium: collaborative study. J AOAC Int . 1994 Mar-Apr;77(2):365-73.
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5. Veenman C, Korver H, Mooijman KA. Improvements in the method for detection of Salmonella spp. In animal faeces. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM report 330300 010. 2007.
6. 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

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 1	Updated layout and content	2022/11

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

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