Instructions for Use

REF - KM0047 **Rappaport-Vassiliadis Soya Broth (RVS)**

Dehydrated Culture Medium

1. Intended Use

KM0047 Rappaport-Vassiliadis Soya Broth (RVS) is for the enrichment and selective isolation of *Salmonella* species [1] from food, dairy, and environmental samples.

2. Composition*

Ingredient	g/L
Soya peptone	4.5
Sodium chloride	7.2
Potassium dihydrogen phosphate	1.26
Di-potassium hydrogen phosphate	0.18
Magnesium chloride (Anhydrous)	13.4
Malachite green	0.036

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

This medium is a modification of the original formulation by Rappaport *et al.* [1] It is designed to use the ability of *Salmonella* species to multiply in lower pH conditions, survive high osmotic pressure, and resist selective pressure from malachite green. The concentration of magnesium chloride in this formulation is optimised [2]. Rappaport-Vassiliadis Soya Broth has been shown to be superior to tetrathionate broth and selenite broth for the isolation of *Salmonella* species from meat products [3]. It can also be used to isolate salmonellae from human faeces without the need for pre-enrichment.

E&O Rappaport-Vassiliadis Soya Broth meets the requirements of ISO 11133 [4], ISO 6579 [5 6] and Harmonised EP/USP/JP [7 8 9].

NB: This formulation will produce a slight exothermic reaction when mixed with water. It is also very hygroscopic.

4. Principle

Soya peptone supplies the required carbon, nitrogen, and vitamins. Potassium dihydrogen phosphate and di-potassium hydrogen phosphate function as buffers to maintain pH. Magnesium chloride raises the osmotic pressure in the medium. Malachite green is an inhibitory substance.

5. Preparation Instructions

Suspend 26.75g/L of dried culture media in deionised/purified water and allow to soak whilst mixing for 10 minutes.

Dispense into final containers and autoclave to sterilise at 115°C for 15 minutes.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Pale green fine powder	Blue liquid
pH	N/A	5.2 ± 0.2

7. Materials Provided

KM0047 can be provided in the formats detailed below. Each tub is labelled with product name, product code, lot number, expiry date, instructions, and appropriate warnings.

Product Code	Product Format
KM0047-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0047-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0047-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

Standard microbiological laboratory materials *e.g.,* autoclave, sterile loops or swabs, collection containers, incubators, and quality control organisms.

9. Specimens

KM0047 Rappaport-Vassiliadis Soya Broth (RVS) is suitable for the testing of the following specimens:

- Food and Environmental: products for human consumption, animal feed and environmental samples

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

10. Test Procedures and Interpretation of results

Allow the media to come to room temperature.

Food and Environmental Samples (according to ISO 6579 [5 6]):

Prepare a homogenised suspension using a ratio of 1:10 of sample to Buffered Peptone Water (E&O BM0082) and incubate aerobically for 16-20 hours at 36 ± 2°C. Subculture the required volumes to Rappaport-Vassiliadis Broth and Muller-Kauffmann Tetrathionate Novobiocin (MKTTn) Broth (E&O BM0947) and incubate aerobically at 41.5°C and 37°C respectively for 24 ± 3 hours.

Using a 10µl loop, inoculate one plate of Xylose Lysine Deoxycholate (XLD) Agar (E&O PP0320) and a secondary selective isolation medium of the user's choice from the Rappaport-Vassiliadis Broth. Repeat for the Muller-Kauffmann tetrathionate novobiocin Broth.

Incubate aerobically at 37 ± 1°C for 21-27 hours. Incubate the secondary medium according to manufacturer's instructions.

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical, serological, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
S. typhimurium (NCTC 12023)	Duplicate plates, one at 30-35°C for 24 ± 3 hours and the other at 41.5 ± 1°C for 24 ± 3 hours	Subplate: Translucent colonies with black centre (ISO, USP)
E. coli (NCTC 12241)	41.5 ± 1°C for 24 ± 3 hours	Subplate: Inhibited (ISO)
P. aeruginosa (NCTC 12903)	41.5 ± 1°C for 24 ± 3 hours	Subplate: Inhibited (ISO)
S. abony (NCTC 6017)	30-35°C for 24 ± 3 hours	Subplate: Translucent colonies with black centre (USP)
E. faecalis (NCTC 12697)	41.5 ± 1°C for 24 ± 3 hours	Subplate: Inhibited (ISO)
S. aureus (NCTC 10788*)*	30-35°C for 24 ± 3 hours	Subplate: Inhibited (USP)

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

12. Performance

To fully verify KM0047 Rappaport-Vassiliadis Soya Broth (RVS) performance, dehydrated culture media samples were used to prepare RVS Broth and tested to assess colony morphology and recovery level when subcultured onto both E&O PP0320 Xylose Lysine Deoxycholate (XLD) Agar and E&O PP0280 Tryptone Soya Agar. Prepared samples were inoculated with 30-150cfu for the target organisms and 10^4-10^5 cfu for the non-target organisms then incubated at 41.5 ± 1°C for 24 ± 3 hours (ISO:11133) and 30-35°C for 24 ± 3 hours (Harmonised USP/EP/JP) as described in the Quality Control table above. All samples of prepared media produced showed expected recovery and morphology of the required test organisms: *Salmonella typhimurium* (NCTC 12023) and *Salmonella abony* (NCTC 6017), and no recovery of the non-target test organisms: *Escherichia coli* (NCTC 12241), *Pseudomonas aeruginosa* (NCTC 12903), *Enterococcus faecalis* (NCTC 12697) and *Staphylococcus aureus* (NCTC 10788). Therefore, it can be concluded that KM0047 Rappaport-Vassiliadis Soya Broth (RVS), meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- Incubation temperature is critical and should be tightly controlled.
- Selective pressures of this medium may inhibit certain salmonella strains (e.g., *S. enteritidis* and *S. typhi*).
- Due to natural variation, some strains may grow poorly on this medium.

14. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSSH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to KM0047 Material Safety Data Sheet.

15. Storage conditions and Shelf life

Store product in the original container with the lid tightly closed at between 10 and 30°C in low humidity conditions away from direct sunlight. Kept under these conditions, the product may be used up to the date of expiry shown on the product label.

Do not use if the product is not free-flowing or displays any sign of colour change, formation of large lumps or hardening of the powder. Additionally, do not use medium if it has been stored inappropriately, the packaging has been damaged or has passed the expiry date.

Dehydrated culture media does not need to be used all at once; replace the cap and ensure that the container is tightly closed and stored according to labelled instructions.

Dispose of in accordance with local and national authority requirements.

16. References

1. Rappaport, F., Konforti, N., and Navon, B. (1956) A New Enrichment Medium for Certain Salmonellae. J. Clin. Pathol., 9(3), 261-266.
2. Vassiliadis, P., Patéraki, E., Papaïconomou, N., Papadakis, J. A., and Trichopoulos, D. (1976) Noueau procédé d'enrichissement de Salmonella [A new procedure of "Salmonella" enrichment (author's transl)]. Ann. Microbiol, 127B (2), 195–200.
3. Vassiliadis, P. (1983) The Rappaport-Vassiliadis (RV) enrichment medium for the isolation of salmonellas: An overview. J. Appl. Microbiol., 54(1), 69-76.
4. ISO (2014) 11133:2014 Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media. Geneva: ISO.
5. ISO (2017) 6579-1:2017 Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella. Geneva: ISO.
6. ISO (2020) 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 s - Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC. Geneva: ISO.
7. Council of Europe (2019) European Pharmacopoeia. 10th ed., Strasbourg: Council of Europe.
8. United States Pharmacopeial Convention (2019) The United States Pharmacopeia USP42, The National Formulary NF37. Rockville, MD: United States Pharmacopeial Convention.
9. The Ministry of Health, Labour and Welfare (2021) The Japanese Pharmacopoeia. 18th ed. Tokyo: Yakuji Nippo Limited.

Version History*

001 18/07/23 - New Document Created

002 19/01/26 – Typographical corrections

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

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TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code		Manufacturer	Use by
Temperature limitation	Σ Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place