
RAPPAPORT-VASSILIADIS SOJA (RVS) BROTH

SELECTIVE ENRICHMENT OF *SALMONELLA*

1 INTENDED USE

Rappaport-Vassiliadis Soja Broth is used for the selective enrichment of *Salmonella* in milk, dairy products, other food products, water and in environmental samples.

The typical composition of the broth corresponds to that defined in the standards NF EN ISO 19250, NF EN ISO 6579-1, NF U47-102 and in the European Pharmacopeia.

2 HISTORY

The composition of the medium was developed by Rappaport following the observation that *Salmonella* was more resistant to hypertonic media than most other enterobacteria. In his experiments, Rappaport showed that magnesium chloride was the most effective of all the salts tested. Selectivity of the medium was increased still further by the addition of malachite green. Vassiliadis subsequently showed that a larger number of *Salmonella* could be recovered by reducing the malachite green content and incubating at 43°C instead of 37°C.

Subsequent studies by Peterz *et al.* showed the effects of incubation temperature and magnesium chloride concentration on the recovery performance of the medium. Finally, van Schothorst and Renaud modified Rappaport-Vassiliadis Broth by replacing the casein peptone with a soy peptone and by incorporating a potassium hydrogenophosphate buffer into the formula, resulting in a greater stability of the medium over time.

3 PRINCIPLES

The high concentration of magnesium chloride and the presence of malachite green reduce the growth of bacteria other than salmonellae.

Growth of *Salmonella* Typhi, *Salmonella* Paratyphi and *Shigella* is somewhat inhibited by malachite green.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

Pour 1 liter de milieu :

- Papaic digest of soja	4,50 g
- Sodium chloride	7,20 g
- Monopotassium phosphate	1,26 g
- Dipotassium phosphate	0,18 g
- Magnesium chloride, anhydrous (*)	13,40 g
- Malachite green (oxalate).....	36,0 mg

pH of the ready-to-use media at 25 °C : 5,2 ± 0,2.

(*) : 13,40 g/L of anhydrous magnesium chloride (molecular weight 95,21) correspond to 28,6 g/L of hexahydrated magnesium chloride (molecular weight 203,3).

5 PREPARATION

- Dissolve 26,6 g of dehydrated media (BK148) in 1 liter of distilled or demineralized water.
- Stir slowly until complete dissolution.
- Dispense in tubes at 10 mL per tube.
- Sterilize in an autoclave at 115 °C for 15 minutes.
- Cool the media to room temperature.

✓ **Reconstitution :**
26,6 g/L

✓ **Sterilization :**
15 min at 115 °C

6 INSTRUCTIONS FOR USE

- Transfer 0,1 mL of pre-enrichment broth was used (Buffered Peptone Water into a 10 mL tube of RVS broth prepared as above or use the ready-to-use tubes (BM074).
- Incubate at 41,5 ± 1 °C for 24 ± 3 hours.

✓ **Inoculation :**
0,1 mL / tube

✓ **Incubation :**
24 h at 41.5 °C

NOTES :

- In dried milk products and cheese, *Salmonella* may be sublethally injured. Incubate the selective enrichment media from these products for an additional 24 h ± 3 h.
- A second selective enrichment broth is cultured in parallel when working in the context of the standardized methods.

7 RESULTS

From each tube, inoculate a plate of XLD agar and a second selective media of the user's choice for *Salmonella*, using a loop of RVS broth.
Confirm characteristic colonies.

8 QUALITY CONTROL

Dehydrated media : bluish powder, free-flowing and homogeneous.

Prepared media : blue, limpid solution.

Typical culture response after 24 hours of incubation at 41,5 °C followed by subculture on XLD or Casein soy agar (NF EN ISO 11133) :

Microorganisms		Growth
<i>Salmonella</i> Enteritidis + <i>Escherichia coli</i> + <i>Pseudomonas aeruginosa</i>	WDCM 00030 WDCM 00013 WDCM 00025	> 10 characteristics colonies on XLD agar
<i>Salmonella</i> Typhimurium + <i>Escherichia coli</i> + <i>Pseudomonas aeruginosa</i>	WDCM 00031 WDCM 00012 WDCM 00025	> 10 characteristics colonies on XLD agar
<i>Escherichia coli</i> <i>Enterococcus faecalis</i>	WDCM 00012 WDCM 00087	≤ 100 colonies on TSA < 10 colonies on TSA

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.

Ready-to-use media in tubes : 2-8 °C.

The expiration dates are indicated on the label..

Prepared media in tubes (*) : 180 days at 2-8 °C.

(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :

500 g bottle BK148HA

Ready to use media in tubes :

50 x 10 mL tubes BM07408

11 BIBLIOGRAPHY

Rappaport, F., Konforti, N., and Navon, B.. 1956. A new enrichment medium for certain salmonellae. Journal of Clinical Pathology, **9** : 261-266.

Vassiliadis, P., Kalapothaki, V., Trichopoulos, D., Papadakis, J.A., and Serie, C.H.. 1979. Recent experience on the use of modified Rappaport's medium (R 10/43°C) for the isolation of *Salmonella*. Quality assurance and quality control of microbiological culture media. Dr Janet E.L. Corry. London, 141-145.

van Schothorst, M., and Renand, A.M.. 1983. Dynamics of *Salmonella* isolation with modified Rappaport medium (R 10). Journal of Applied Bacteriology, **54** , 209-215.

van Schothorst, M., Renaud, A., and van Beek, C.. 1987. Salmonella isolation using RVS broth and MLCB agar. Food Microbiology, **4** , 11-18.

Peterz, M., Wiberg, C., and Norberg, P. 1989. The effect of incubation temperature and magnesium chloride concentration on growth of salmonella in home-made and in commercially available dehydrated Rappaport-Vassiliadis broths. Journal of Applied Bacteriology, **66** : 523-528.

NF U 47-102. Janvier 2008. Méthodes d'analyse en santé animale. Isolement et identification de tout sérovar ou de sérovar(s) spécifique(s) de salmonelles chez les mammifères.

NF EN ISO 19250. Juin 2013. Qualité de l'eau. Recherche de *Salmonella* spp.

Pharmacopée européenne. Chapitre 2.6.13. Recherche de microorganismes spécifiques.

NF EN ISO 11133. Juillet 2014. Microbiologie des aliments, des aliments pour animaux et de l'eau - Préparation, production, stockage et essais de performance des milieux de culture (Tirage 2 (2016-01-01)).

NF EN ISO 6579-1. Avril 2017. Microbiologie de la chaîne alimentaire - Méthode horizontale pour la recherche, le dénombrement et le sérotypage des *Salmonella* - Partie 1 : recherche des *Salmonella* spp..

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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