

RAPPAPORT VASSILIADIS SOYA BROTH (RVS BROTH)**TM 1282**

For selective enrichment of Salmonellae

Composition

Ingredients	Gms/Ltr.
Magnesium chloride (anhydrous)	13.58
Sodium chloride	7.20
Soya peptone	4.50
Potassium phosphate, monobasic	1.26
Sodium phosphate, dibasic	0.18
Malachite green	0.036

* Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°C and protect from direct Sunlight.

Instructions for Use

Dissolve 26.75gms in 1000ml distilled water. Gently heat to boiling with gentle swirling and dissolve the medium completely. Dispense 10 ml amount into screw cap glass tubes. Sterilize to autoclave at 115°C for 15 minutes. Cool the medium to 25°C.

Appearance: Dark turquoise, clear, may have light ppt.

pH (at 25°C): 5.2 ± 0.2

Principle

RAPPAPORT VASSILIADIS SOYA BROTH (RVS BROTH) used for selective enrichment of Salmonellae. 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. Finally, van Schothorst and Renaud modified Rappaport Vassiliadis Broth by replacing the Casein peptone with a Soya peptone as the carbon and nitrogen sources for general growth requirements. By incorporating a Monopotassium phosphate buffer into the formula, resulting in a greater stability of the medium over time. Selectivity of the medium was increased still further by the addition of malachite green. Malachite Green is inhibitory to organisms other than *Salmonella* spp. The low pH of the medium, combined with the presence of Malachite Green and Magnesium Chloride, select for the highly resistant *Salmonella* spp.

Method:- Transfer 0.1 ml of inoculum into a tube of 10 ml of medium prepared (respect the 1:100 ratio). Inoculum generally constitutes a pre-enrichment medium: **Buffered Peptone Water (TM 307)** - Incubate . and incubate at 35°C for 16 – 20 hours. Carry out isolation in several selective media for *Salmonella* sp., with a platinum loop. Inoculate 0.1 ml of the pre-enrichment Buffered Peptone Water to 10 ml of Rappaport - Vassiliadis Soya Peptone Broth and incubate at 41.5 ± 0.5°C for 21 - 27 hours. Subculture onto confirmatory medium, including XLD Agar at 35 ± 2°C. Examine for growth after 18 – 24 hour incubation.

Interpretation

Cultural characteristics observed after inoculating (10^3 CFU/ml), on incubation at 41.5°C for 24 hours, followed by subculture in XLD agar, incubated at $35 \pm 2^\circ\text{C}$, and examined for growth after 18 – 24 hours.

Microorganisms	ATCC	Inoculum (CFU/ml)	Recovery on XLD Agar
<i>Salmonella typhimurium</i>	14028	10^3	Growth, red colonies, black centers
<i>Salmonella arizonae</i>	13314	10^3	Growth, red colonies, black centers
<i>Pseudomonas aeruginosa</i>	27853	10^3	Suppressed, red colonies
<i>Escherichia coli</i>	25922	10^3	Inhibited to suppressed, yellow colonies

Reference

1. RAPPAPORT, F., KONFORTI, N., and NAVON, B. A new enrichment medium for certain salmonellae. *Journal of Clinical Pathology*, 9: 261-266. (1956).
2. VASSILIADIS, P., KALAPOTHAKI, V., TRICHOPOULOS, D., PAPADAKIS, J.A., and SERIE, C.H. 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. (1979).