Pre-enrichment

2. Using aseptic technique, transfer the cottonwool plug or the pad to 100 mL of a suitable pre-enrichment medium such as Buffered Peptone Water.
3. Incubate at 37 ± 0.5°C for 18-24 hours.

Selective Enrichment

4. Inoculate 10 mL of Rappaport-Vassiliadis R10 Broth with 0.1 mL of the pre-enrichment culture. Inoculate 10 mL of Muller-Kauffman Tetrathionate Broth with 1 mL of the pre-enrichment culture.
5. Incubate Rappaport-Vassiliadis R10 Broth at 41.5 ± 0.5°C. Incubate Muller- Kauffman Tetrathionate Broth at 42 ± 1°C for 48 hours.

Expected Results

6. After incubation, subculture both selective enrichment broths to Brilliant Green Agar and XLD Agar. Incubate at 35 ± 2°C for 18-24 hours.
7. Examine for typical *Salmonella* colonies. Confirm identification of isolates by biochemical and serologic tests.

Milk and Foods

For isolating *Salmonella* (other than *S.* Typhi) from milk and milk products,[4] raw flesh foods, highly contaminated foods and animal feeds:[5]

Pre-enrichment

1. Add 25 g or a 25 mL sample of the specimen to 225 mL of pre-enrichment medium. Consult appropriate references for the type of product being tested.[4,5]
2. Incubate at 35 ± 2°C for 20-24 hours[5] or at 37°C for 16-20 hours,[4] depending on the referenced procedure being followed.

Selective Enrichment

3. Inoculate 10 mL of Rappaport-Vassiliadis R10 Broth with 0.1 mL of pre-enrichment culture. Inoculate 10 mL of another selective enrichment medium such as Tetrathionate Broth or Selenite Cystine Broth with the recommended amount of pre-enrichment culture.[4,5]
4. Incubate Rappaport-Vassiliadis R10 Broth at 41.5 ± 0.5°C[4] for 24 ± 2 hours or at 42 ± 0.5°C for 22-24 hours.[5] Incubate the other selective enrichment broths appropriately.

Expected Results

5. After incubation, subculture Rappaport-Vassiliadis R10 Broth and the other selective enrichment broths to selective agar media and incubate at 35 ± 2°C for 24 ± 2 hours[4] or for 18-24 hours.[5]
6. Examine for typical *Salmonella* colonies. Confirm identification of isolates by biochemical and serologic tests.[4,5]

Limitation of the Procedure

The combined inhibitory factors of this medium (malachite green, magnesium chloride, low pH) may inhibit certain *Salmonella*, such as *Salmonella* Typhi and *S.* Paratyphi A. Isolation techniques should include a variety of enrichment broths and isolation media.

References

1. Rappaport, Konforti and Navon. 1956. J. Clin. Pathol. 9:261.
2. Vassiliadis, Trichopoulos, Kalandidi and Xirouchaki. 1978. J. Appl. Bacteriol. *44*:233.
3. Peterz, Wiberg and Norberg. 1989. J. Appl. Bacteriol. *66*:523.
4. International Organization for Standardization. 2001. Milk and milk products – detection of *Salmonella*. ISO 6785/IDF 93:2001. ISO, Geneva, Switzerland.
5. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.

Availability

Difco™ Rappaport-Vassiliadis R10 Broth

IDF ISO USDA

Cat. No. 218581 Dehydrated – 500 g

Europe

Cat. No. 257257 Prepared Tubes, 10 mL – Ctn. of 50

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Rappaport Vassiliadis Salmonella (RVS) Soy Broth

Intended Use

Rappaport Vassiliadis Salmonella (RVS) Soy Broth is used for selectively enriching *Salmonella* in food and environmental samples.

Meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

Rappaport et al.[4] formulated an enrichment medium for *Salmonella* that included very high amounts of malachite green and magnesium chloride as inhibitors. The original Rappaport medium was developed for the enrichment of *S. paratyphi* and other serotypes that were known to be relatively resistant to brilliant green. In addition, magnesium chloride was found to counteract the toxic effect of the dye for *Salmonella*.[5] Vassiliadis et al. modified the formulation by reducing the concentration of the malachite green to one third.[6]

Van Schothorst and Renaud reported that using soy peptone instead of animal peptone improved recovery rates of *Salmonella*.[7] Similar results were obtained in several other studies.[8-11]

Vassiliadis et al. recommended incubation of RV media at 43°C for maximum selectivity.[6] Any deviation above 43°C may be lethal for *Salmonella*. Later, work by Peterz showed that incubation at 41.5 ± 0.5°C for 24 hours improved recovery of *Salmonella* spp.[12]

RVS Soy Broth is a selective enrichment medium that is used following pre-enrichment of a sample in a suitable pre-enrichment medium. It has gained approval for use in analyzing milk

and milk products,[13] food,[14,15] animal feed,[15] and nonsterile pharmaceutical products.[1] This medium selectively enriches for salmonellae because bacteria, including other intestinal bacteria, are typically inhibited by malachite green, high osmotic pressure and/or low pH. *S.* Typhi and *S.* Paratyphi A are sensitive to malachite green and may be inhibited.

Principles of the Procedure

RVS Soy Broth contains soy peptone as the carbon and nitrogen source for general growth requirements. Magnesium chloride raises the osmotic pressure in the medium. Sodium chloride maintains osmotic balance. Dipotassium phosphate and potassium dihydrogen phosphate are buffering agents. Malachite green is inhibitory to organisms other than salmonellae. The low pH of the medium, combined with the presence of malachite green and magnesium chloride, helps to select for the highly resistant *Salmonella* spp.

Formula

Difco™ RVS Soy Broth

Approximate Formula* Per Liter		
Soy Peptone	4.5	g
Magnesium Chloride (anhydrous)	13.5	g
Sodium Chloride	9.0	g
Dipotassium Phosphate	0.03	g
Potassium Dihydrogen Phosphate	1.45	g
Malachite Green	36.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

NOTE: Formula is further adjusted from the *USP* formulation as follows. Since magnesium chloride hexahydrate contains too much water to be effectively used in the manufacture of dehydrated culture media, magnesium chloride anhydrous (without water) is substituted. The actual amount of magnesium chloride (minus water) is the same. However, the use of the anhydrous magnesium requires slight adjustments in the rest of the formulation. None of these slight changes affect performance, as is indicated on the Certificate of Analysis, which shows that harmonized *USP/EP/JP* growth promotion criteria are met per requirements for the Microbiological Examination of Nonsterile Products.

Directions for Preparation from Dehydrated Product

1. Suspend 28.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Dispense 10 mL amounts into suitable containers.
4. Autoclave at 115°C (10 psi pressure) for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

Follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[1,13-15]

User Quality Control

Identity Specifications

Difco™ RVS Soy Broth

Dehydrated Appearance:	Pale green to green, free-flowing, homogeneous.
Solution:	2.85% solution, soluble in purified water upon gentle heating. Solution is blue, clear.
Prepared Appearance:	Blue, clear.
Reaction of 2.85% Solution at 25°C:	pH 5.2 ± 0.2

BBL™ RVS Soy Broth (prepared)

Appearance:	Blue and clear.
Reaction at 25°C:	pH 5.2 ± 0.2

Cultural Response

Difco™ RVS Soy Broth

Prepare the medium per label directions. Inoculate and incubate at 30-35°C for 24 hours. After incubation, subculture to XLD Agar plates and incubate at 30-35°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	<100	Growth
Staphylococcus aureus	6538	>100	No growth

BBL™ RVS Soy Broth (prepared)

Inoculate and incubate at 30-35°C for 18-24 hours. After incubation, subculture to XLD Agar plates and incubate at 30-35°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10-100	Growth
Staphylococcus aureus	6538	>100	No growth

Procedure

Refer to appropriate references for details on test methods using RVS Soy Broth.[1,13-15] Inoculate tubes with the test sample and incubate as instructed in appropriate references.[1,13-15]

Expected Results

Examine selective plates for typical *Salmonella* colonies. Confirm identification of isolates by biochemical and/or serological tests as directed in appropriate references.

Limitation of the Procedure

The combined inhibitory factors of this medium (malachite green, magnesium chloride, low pH) may inhibit certain *Salmonella*, such as *S.* Typhi and *S.* Paratyphi A. Isolation techniques should include a variety of enrichment broths and isolation media.

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed., online. Japanese Ministry of Health, Labour and Welfare.
4. Rappaport, Konforti and Navon. 1956. J. Clin. Pathol. 9:261.
5. Rappaport and Konforti. 1959. Appl. Microbiol. 7:63.
6. Vassiliadis, Paternaki, Papaiconomon, Papadakis and Trichopoulos. 1976. Ann. Microbiol. Inst. Pasteur. *127B*:195.
7. Van Schothorst and Renaud. 1983. J. Appl. Bacteriol. 54:209.
8. McGibbon, Quail, and Fricker. 1984. Int. J. Food Microbiol. 1:171.
9. Fricker and Girdwood. 1985. J. Appl. Bacteriol. 58:343.
10. Fricker, Quail, McGibbon, and Girdwood. 1985. J. Hyg. Cambridge. 95:337.
11. Quail, McGibbon and Fricker. 1986. J. Hyg. Cambridge. 96:425.
12. Peterz, Wiberg and Norberg. 1989. J. Appl. Bacteriol. 66:523.
13. International Organization for Standardization. 2001. Milk and milk products – Detection of *Salmonella* spp. ISO 6785, IDF 93, 2001-05-15. International Organization for Standardization, Geneva, Switzerland.
14. United Stated Department of Agriculture. 2008. Microbiology laboratory guidebook, online. MLG 4.04, Isolation and identification of *Salmonella* from meat, poultry and egg products. USDA, Food Safety and Inspection Service, Office of Public Health Science, Athens, Ga.
15. International Organization for Standardization. 2002. Microbiology of food and animal feeding stuffs – horizontal method for the detection of *Salmonella* spp. ISO 6579, 2002-07-15. International Organization for Standardization, Geneva, Switzerland.

Availability

Difco™ RVS Soy Broth

CCAM EP ISO JP USDA USP

Cat. No. 214943 Dehydrated – 500 g†

BBL™ RVS Soy Broth

CCAM EP ISO JP USDA USP

Cat. No. 215199 Prepared Tubes, 10 mL – Pkg. of 10†

† QC testing performed according to USP/EP/JP performance specifications.

Regan-Lowe Charcoal Agar
Regan-Lowe Charcoal Agar without Cephalexin

Intended Use

Regan-Lowe Charcoal Agar is a selective medium used for isolation of *Bordetella pertussis* from clinical specimens. Regan-Lowe Charcoal Agar without Cephalexin is used for the cultivation of *B. pertussis* from clinical specimens and for subcultures of the bacterium.

Summary and Explanation

Regan-Lowe Charcoal Agar plates are used in clinical laboratories for the isolation of *Bordetella pertussis*, the etiologic agent of whooping cough, from nasopharyngeal swabs and other sources of pharyngeal exudate. This medium was developed by Regan and Lowe as a transport medium for whooping cough specimens, but proved useful as an enrichment medium for the selective isolation of *B. pertussis* and *B. parapertussis*. It consists of charcoal agar as a basal medium supplemented with cephalexin to inhibit bacteria indigenous to the nasopharynx and defibrinated horse blood to support the growth of *Bordetella* species.[1-3]

Use of the medium without cephalexin in parallel with Regan-Lowe Charcoal Agar is recommended, since a few strains (<10%) of *B. pertussis* will not grow on selective plates; also the nonselective medium is used for subcultures to obtain a larger amount of growth for additional testing, such as agglutination or immunofluorescence testing.[3,4]

The medium in 10 mL prepared tubes (deeps) with screw-caps offers a longer shelf-life than the pre-poured plated medium.

To prepare the medium from the agar base, 10% horse blood is added and cephalexin can be added to achieve selectivity.

Principles of the Procedure

Beef extract and enzymatic digest of gelatin provide the amino acids and other complex nitrogenous substances necessary

User Quality Control

Identity Specifications

BBL™ Regan-Lowe Charcoal Agar Base

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	5.1% solution, soluble in purified water upon boiling. Solution is charcoal black, homogeneous, opaque.
Prepared Appearance:	Charcoal black, homogeneous, opaque.
Reaction of 5.1% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

BBL™ Regan-Lowe Charcoal Agar Base

Prepare the medium per label directions. Inoculate with fresh broth cultures diluted 1:10 and incubate at 35 ± 2°C for 7 days.

ORGANISM	ATCC™	RECOVERY
Bordetella pertussis	9797	Good
Bordetella parapertussis	15311	Good

Bordetella pertussis
ATCC™ 9797