IFU

Instructions for Use

RAPPAPORT-VASSILIADIS R10 BROTH

Cat. no. K167	Rappaport-Vassiliadis R10 Broth, 16x125mm Tube, 10ml	20 tubes/box

INTENDED USE

Hardy Diagnostics Rappaport-Vassiliadis R10 Broth is recommended for the selective enrichment of *Salmonella* spp. from food and sewage polluted water.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Rappaport Medium was initially developed by Rappaport, et al., in 1956 as an alternative to Tetrathionate Broth for the enrichment of *Salmonella*.(1) A high concentration of magnesium chloride was included to inhibit growth of *Proteus* and *Escherichia coli*. The addition of malachite green inhibits coliforms. In addition, the low pH inhibits microorganisms other than *Salmonella*. In 1976, Vassiliadis, et al., described a modification of Rappaport Medium called R10. This formula featured a reduced amount of malachite green.(2) Hardy Diagnostics Rappaport-Vassiliadis R10 Broth is based on this formula.

FORMULA

Ingredients per liter of deionized water:*

Magnesium Chloride	13.4gm
Sodium Chloride	7.2gm
Pancreatic Digest of Casein	4.54gm
Potassium Phosphate	1.45gm
Malachite Green	36.0mg

Final pH 5.1 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC away from direct light. Media should not be used if there are any signs of contamination, deterioration, discoloration, or if the expiration date has passed.Product is light and temperature sensitive; protect from light, excessive heat, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The

product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Consult listed references for information regarding collection of specimens suspected of containing *Salmonella*.[3,5,6,8-10]

Pre-Enrichment
Inoculate the sample to be tested into Buffered Peptone Water or Lactose Broth and incubate for 18 to 24 hours at 35 to 37ºC.

Selective Enrichment
Inoculate the Rappaport-Vassiliadis R10 Broth with 0.1ml of the pre-enrichment culture. Inoculate a tube of Tetrathionate Broth in the same manner. Incubate The Rappaport-Vassiliadis R10 Broth at 41.5ºC for 48 hours. The Tetrathionate Broth is incubated for 48 hours at 42ºC.

Subculture
Subculture each enrichment broth to Brilliant Green Agar and XLD Agar and incubate at 37ºC. for 48 hours. After incubation, examine plates for colonies typical of *Salmonella* . Suspect *Salmonella* colonies should be identified by biochemical and serological tests. See listed references.[3,5,6,8-10]

For more detailed procedural information, consult listed references.[3,5,6,8-10]

INTERPRETATION OF RESULTS

Consult listed references for information on interpretation of growth in Rappaport-Vassiliadis R10 Broth.[3,5,6,8-10]

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Rappaport-Vassiliadis R10 Broth is a selective enrichment for *Salmonella* . Other biochemical and/or serological tests must be performed for complete identification. See listed references.[3,5,6,8-10]

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, slides, staining supplies, other culture media, microscopes, incinerators, and incubators, etc., as well as other biochemical and serological reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Salmonella enterica ATCC® 14028	A	18-24hr	35°C	Aerobic	Growth when subcultured to XLD Agar
Escherichia coli ATCC® 25922	B	18-24hr	35°C	Aerobic	Inhibited when subcultured to XLD Agar

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Rappaport-Vassiliadis R10 Broth should appear clear, and blue-green in color.

REFERENCES

1. Rappaport, F., et al. 1956. *J. Clin. Path.*; 9:261-266.

2. Vassiliadis, P., et al. 1978. *J. Appl. Bact.*; 44:233-239.

3. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

4. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

5. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I & II. American Society for Microbiology, Washington, D.C.

6. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

7. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams &

Wilkins, Baltimore, MD.

8. FDA. 1995. *Bacteriological Analytical Manual*, 8th ed. FDA.

9. Marshall, R.T., ed. 1992. *Standard Methods for the Examination of Dairy Products*, 16th ed. APHA, Washington, D.C.

10. Vanderzant, C. and D.F. Splittstoesser, (ed.). 1992. *Compendium of Methods for the Microbiological Examination of Foods*, 3rd ed. APHA, Washington, D.C.

11. Greenberg, A.E., et al., (ed.). 1992. *Standard Methods for the Examination of Water and Wastewater*, 18th ed. APHA, Washington, D.C.

12. *U.S. Pharmacopeia*, 22nd rev. 1990. U.S. Pharmacopeial Convention, Rockville, MD.

13. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22-A2, Vol. 16, No. 16. 1996. Clinical Laboratory Standards Institute (CLSI - formerly NCCLS), Villanova, PA.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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