Technical Data Sheet

MSRV (Modified Semi-Solid RAPPAPORT-VASSILIADIS) Medium (Base) acc. ISO 6579

Ordering number: 1.09878.0500

For the selective enrichment and presumptive detection of *Salmonella* from food and animal feed, animal faeces and environmental samples from primary production stage.

This culture medium complies with the specifications given by EN ISO 6579/Amd 1 and EN ISO/FDIS 6579-1.

Mode of Action

This medium is a semi-solid modification of Rappport-Vassiliadis enrichment broth and is used as a motility enrichment in Petri dishes (de Smedt et. al. 1986). It is a rapid and sensitive medium for the isolation of *Salmonella* spp. and can be seeded after directly after pre-enrichment.

Its efficiency is due to the ability of salmonellae to migrate into the semi-solid medium thus forming opaque halos of growth. The motility of other microorganisms is largely inhibited by the elective agents magnesium chloride, malachite green and novobiocin and the enhanced incubation temperature of 41.5 °C.

According to EN ISO 6579/Amd 1 and EN ISO FDIS 6579-1, MSRV medium contains 10 ml/l novobiocin whilst the original composition of MSRV medium contains 20 mg/l (de Smedt et al. 1986).

Studies have shown larger migration zones on MSRV medium with a lower concentration of novobiocin and the (negative) influence of novobiocin on bacterial motility (Veenman et al. 2007).

Because the detection principle is based on motility, this MSRV medium is not appropriate for the detection of non-motile *Salmonellae*.

EN ISO 6579/Amd 1 describes the usage of MSRV medium for the detection of *Salmonellae* in samples from the primary production stage. EN ISO FDIS 6579-1 gives the choice between using RVS broth or MSRV semi-solid medium for food, animal feed samples and for environmental samples from the food production area, it includes the usage of MSRV medium for samples from primary production stage.

Typical Composition

Specified by ISO 6579/ Amd 1, ISO FDIS 6579-1		MSRV (Modified Semi-Solid RAPPAPORT-VASSILIADIS) Medium (Base) acc. ISO 6579	
Enzymatic Digest of Animal and Plant Tissue	4.6 g/l	Enzymatic Digest of animal and plant tissue	4.6 g/l
Acid Hydrolysate of Casein	4.6 g/l	Casein Hydrolysate	4.6 g/l
NaCl	7.3 g/l	NaCl	7.3 g/l
KH_2PO_4	1.5 g/l	KH_2PO_4	1.5 g/l
$MgCl_2$, anhydrous	10.9 g/l	$MgCl_2$, anhydrous	10.9 g/l
Malachite Green Oxalate	0.04 g/l	Malachite Green Oxalate	0.04 g/l
Agar	2.7 g/l	Agar-Agar*	2.7 g/l
Water	1000 ml/l	Water	n/a
Supplement added after heating			
Novobiocin Sodium Salt	0.01 g/l	Novobiocin Sodium Salt	0.01 g/l
pH at 25°C	5.2 (5.1 - 5.4)	pH at 25°C	5.1 - 5.4

* Agar-agar is equivalent to other different terms of agar.

Preparation

Dissolve 15.8 g in 500 ml of purified water. Heat in boiling water, and agitate frequently until completely dissolved. **Do not autoclave!**

Dissolve the lyophilisate of one vial of MSRV Selective Supplement (article number 1.09874.0010) by adding 1 ml of sterile, purified water. Add the solution to the medium cooled to 45-50 °C.

For working according to EN ISO 6579 use 0.5 ml of the MSRV Selective Supplement solution. Mix gently and pour plates up to a volume of 15-20 ml in dishes with a diameter of 90 mm.

Allow the medium to solidify before moving and handle with care. Store the plates with surface upwards and do not invert the plates as the semi-solid medium is too liquid to do so.

Any plates in which the semi-solid medium has liquefied or fragmented shall not be used.

The prepared medium is clear and blue.

Immediately before use and only if visible moisture is apparent, dry the surface of the medium plates carefully, for example, by placing them with the lids off and the medium surface upwards in a laminar flow cabinet. Take care not to overdry the medium.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Allow the MSRV medium to equilibrate at room temperature if it was stored at a lower temperature.

Acc. to EN ISO 6579/Amd 1 and EN ISO FDIS 6579-1, inoculate MSRV plates with 3 drops of the culture obtained in the pre-enrichment (Buffered Peptone Water). The 3 drops should total 0.1 ml and should be placed separately and equally spaced on the surface of the medium.

Minimize the transfer of particulate material from the pre-enrichment into the selective enrichment medium.

Incubate the inoculated MSRV medium under aerobic conditions, e.g. acc. to EN ISO 6579 40.5-42.5 °C for 21-27 h. **Do not invert the plates**. Care should be taken that the maximum allowed temperature (42.5 °C) is not exceed.

Positive MSRV medium will show a grey-white, turbid zone extending out from the inoculated drop.

From the positive growth obtained on MSRV medium, determine the furthest point of opaque growth from the inoculation points and dip a 1 µl-loop just inside the border of the opaque growth. Withdraw the loop ensuring that no large lumps of MSRV medium are extracted. Inoculate the surface of a petri dish containing the first selective plating-out medium so that well-isolated colonies will be obtained. Proceed in the same way with the second selective plating medium, for details see EN ISO 6579/Amd 1 and EN ISO FDIS 6579-1.

According to EN ISO/FDIS 6579-1, for some products it may be necessary to incubate the selective enrichment medium for an additional 24 h, then follow the same plating-out procedure as described above.

For samples from the primary production stage, negative MSRV plates have to be incubated for a further 21-27 h. Perform the selective plating procedure, if after 48 h incubation, these MSRV plates become positive.

According to EN ISO/FDIS 6579-1, it is permissible to store the selective enrichment after incubation at +2 °C to +8 °C for a maximum of 72 h.

Storage

Store at +15 °C to +25 °C, dry and tightly closed. Do not use clumped or discolored medium. Protect from UV light (including sun light). For *in vitro* use only.

According EN ISO 6579/Amd and EN ISO FDIS 6579-1, self-prepared plates medium can be stored **with surface upwards** at +2 °C to +8 °C in the dark and protected against evaporation for up to two weeks.

Quality Control

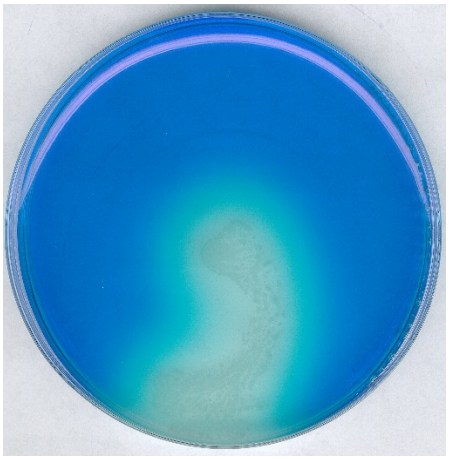
Function	Control strains	Incubation	Method of control	Expected results
Productivity	*Salmonella* Typhimurium ATCC® 14028	2x 21-27 h at 40.5-42.5 °C	Qualitative	Grey-white, turbid zone extending out from inoculated drop. After 24-48 h, the turbid zone will be (almost) fully migrated over the plate.
	Salmonella Enteritidis ATCC® 13076			
Selectivity	*Escherichia coli* ATCC® 8739	2x 21-27 h at 40.5-42.5 °C	Qualitative	Possible growth at the place of the inoculated drop without a turbid zone.
	Escherichia coli ATCC® 25922			
	Enterococcus faecalis ATCC® 19433			No growth
	Enterococcus faecalis ATCC® 29212			
	Citrobacter freundii ATCC® 8090			

Please refer to the actual batch related Certificate of Analysis.

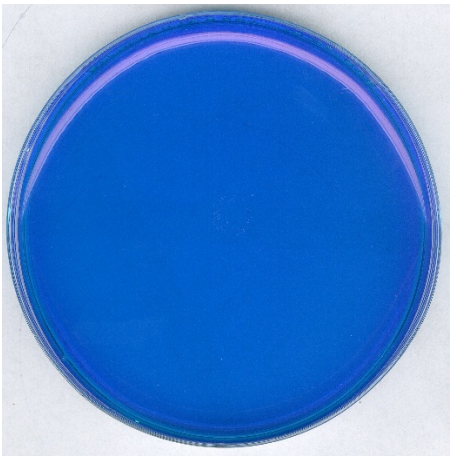
Performance test as MSRV medium with 10 mg/l Novobiocin sodium salt in accordance with EN ISO 6579/Amd 1:2007 and EN ISO/FDIS 6579-1:2015.

Performance test as MSRV medium with 10 mg/l and 20 mg/l Novobiocin sodium salt in accordance with the current version of EN ISO 11133.

Both performance tests are indicated on the product's Certificate of Analysis.



Salmonella Typhimurium ATCC® 14028



Citrobacter freundii ATCC® 8090

Literature

De Smedt, J.M., Bolderdijk, R.F., Rappold, H. and Lautenschlaeger, D. (1986): Rapid *Salmonella* detection in foods by motility enrichment on a modified semi-solid Rappaport-Vassiliadis Medium. J. Food Prot. **49**: 510-514.

De Smedt, J.M. and Bolderdijk, R.F. (1987): Dynamics of *Salmonella* isolation with modified semi-solid Rappaport-Vassiliadis Medium. J. Food Prot. **50**: 658-661.

De Smedt, J.M. and Bolderijk, R.F. (1990): Collaborative study of the international office of cocoa, chocolate and sugar confectionery on the use of motility enrichment for *salmonella* detection in cocoa and chocolate. J. Food Prot. **55**: 659-664.

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. EN ISO 6579:2002.

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. -- Amendment 1: Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage. EN ISO 6579:2002/Amd 1:2007.

ISO International Standardisation Organisation. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Horizontal method for the detection of *Salmonella* spp. EN ISO/FDIS 6579-1:2015.

ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. EN ISO 11133:2014.

Mooijman, K.A. (2012): Culture media for the isolation of *Salmonella*. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds). pp. 261-286. Royal Society of Chemistry, Cambridge, UK.

Veenman, C., Korver, H. and Mooijman, K.A. (2007): Improvements in the method for detection of *Salmonella* spp. In animal faeces. National Institute for Public Helath and the Environment, Bilthoven, the Netherlands. RIVM report 330300 010. http://www.rivm.nl/bibliotheek/rapporten/330300010.pdf.

Ordering Information

Product	Cat. No.	Pack size	Other pack sizes available
MSRV (Modified Semi-solid RAPPAPORT-VASSILIADIS) Medium (Base) acc. ISO 6579	1.09878.0500	500 g	
MSRV Selective Supplement	1.09874.0010	10 x 1 vial	
ReadyTube™ 12 MSRV Medium ISO 6579	1.46622.0100	100 x 12 ml	
GranuCult™ Buffered Peptone Water acc. ISO 6579, ISO 21528, ISO 22964, FDA-BAM and EP	1.07228.0500	500 g	5 kg, 25 kg
Readybag® Buffered Peptone Water acc. ISO 6579, ISO 21528, ISO 22964, FDA-BAM and EP, 5,7 g, irradiated	1.02448.0060	60 bags	60 bags x 29 g 35 bags x 86 g

Product	Cat. No.	Pack size	Other pack sizes available
ReadyTube™ 9 BPW ISO 6579, 6887, 21528	1.46142.0020	20 x 9 ml	100 x 9 ml, 6 x 225 ml, 6 x 1000 ml, 1 x 2000 ml
GranuCult™ MKTTn (MULLER-KAUFFMANN Tetrathionate Novobiocin) Broth (Base) acc. ISO 6579	1.05878.0500	500 g	
Potassium Iodide	1.05043.0250	250 g	
Iodine resublimed	1.04761.0100	100 g	
GranuCult™ RVS (RAPPAPORT-VASSILIADIS-Soya) Broth (Base) acc. ISO 6579	1.07700.0500	500 g	
Novobiocin Sodium Salt	N6160-1-G	1 g	5 g, 25 g
ReadyTube™ 10 RVS Broth ISO 6579	1.46694.0020	20 x 10 ml	100 x 10 ml
GranuCult™ XLD (Xylose Lysine Deoxycholate) Agar acc. ISO 6579	1.05287.0500	500 g	
ReadyPlate™ XLD Agar ISO 6579	1.46751.0020	20 x 90 mm	
RAMBACH® Agar	1.07500.0001	4 x 250 ml	4 x 1000 ml, 4 x 50 l
RAMBACH® Agar ready-to-use	1.46719.0020	20 x 90 mm	100 x 90 mm
Singlepath® Salmonella	1.04140.0001	25 test	
Bismuth Sulfite Agar acc WILSON-BLAIR	1.05418.0500	500 g	
Triple Sugar Iron Agar	1.03915.0500	500 g	
Urea Agar (Base) acc CHRISTIANSEN	1.08492.0500	500 g	

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