TECHNICAL DATA SHEET

MSRV MEDIUM

DETECTION OF MOTILE *SALMONELLA*

1 INTENDED USE

Modified Semi-Solid Rappaport-Vassiliadis Agar (MSRV) is a selective medium historically used for the isolation of *Salmonella* in chocolate and other food products. It is also widely used in animal health: in particular with mammals, poultry and in animal production facilities. It is also recommended for us in the detection of motile *Salmonellae* in animal fecal matter and in environmental samples in the context of primary animal production.

This medium is not recommended for immobile *Salmonellae* (*Salmonella* Gallinarum et Pullorum).

The typical composition responds to that defined in the standards NF EN ISO 6579-1/A1, NF U47-100, NF U47-101 and NF U47-102.

2 HISTORY

The composition of the medium, developed by De Smedt *et al.*, is derived from that of Rappaport-Vassiliadis Broth, made semi-solid by adding a small quantity of agar. Its selectivity has been modified by lowering the level of magnesium chloride and the addition of novobiocin at 10 mg/L.
MSRV (ISO 6579) is used in the protocol for detection of *Salmonella* in the poultry industry (animal fecal material and environmental samples), particularly in hatcheries and farms as well as for *Salmonella* detection with mammals.

3 PRINCIPLES

Bacteriological efficacy is based on the capacity of salmonellae to selectively migrate in the medium.

The medium is not appropriate for immobile salmonellae (*Salmonella* Gallinarum, *Salmonella* Pullorum). If the presence of these strains is suspected, the cultures obtained with preenrichment media must be transferred to appropriate media using conventional standardized procedures.

The high concentration of magnesium chloride and the presence of malachite green inhibit contaminating bacteria.

The addition of novobiocin inhibits most Gram-positive bacteria and avoids the development of *Proteus*.

Salmonella produce an opaque halo, a reflection of growth.

4 TYPICAL COMPOSITION

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

For 1 liter of media:

- Enzymatic digest of animal and plant tissues ..... 4,6 g
- Acid hydrolysate of Casein ..... 4,6 g
- Sodium chloride ..... 7,3 g
- Monopotassium phosphate ..... 1,5 g
- Magnesium chloride, anhydrous ..... 10,9 g
- Malachite green (oxalate) ..... 0,04 g
- Novobiocin ..... 0,01 g
- Bacteriological agar ..... 2,70 g

pH of the ready-to-use media at 25 °C: 5,1-5,4.

Note: This formula corresponds to the completed formula described in note 2 of paragraph B.4.6.2 Preparation on page 8 of amendment A1 of standard NF EN ISO 6579-1.

For 31,7 g of dehydrated media BK191

- Enzymatic digest of plant and animal tissue ..... 4,6 g
- Acid hydrolysate of casein ..... 4,6 g
- Sodium chloride ..... 7,3 g
- Monopotassium phosphate ..... 1,5 g
- Magnesium chloride, anhydrous ..... 10,9 g
- Malachite green (oxalate) ..... 0,04 g
- Novobiocin ..... 0,01 g
- Bacteriological agar ..... 2,70 g

For a vial of supplement BS056
- Novobiocin ..... 40 mg

For 31,6 g of dehydrated base media BK134

- Enzymatic digest of plant and animal tissues ..... 4,6 g
- Acid hydrolysate of casein ..... 4,6 g
- Sodium chloride ..... 7,3 g
- Monopotassium phosphate ..... 1,5 g
- Magnesium chloride, anhydrous ..... 10,9 g
- Malachite green (oxalate) ..... 0,04 g
- Bacteriological agar ..... 2,70 g

For a vial of supplement BS033
- Novobiocin ..... 10 mg

5 PREPARATION

Preparation from dehydrated media BK191

- Dissolve 31,7 g of complete dehydrated media (BK191) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling with constant agitation, stirring until complete dissolution.
- Do not autoclave.
- Cool and maintain the media in a molten state at 44-47 °C.
- Pour into sterile Petri plates and let solidify on a cold, flat surface.
- Do not invert the plates.

✓ **Reconstitution:**
31,7 g/L

✓ **Sterilization:**
Do not autoclave

Preparation from base dehydrated media (BK134) and supplement Novobiocin

- Dissolve 31,6 g of the base dehydrated media (BK134) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling with constant agitation, stirring until complete dissolution.
- Do not autoclave.
- Cool and maintain the media in a molten state at 44-47 °C.
- Reconstitute the 10 mg Novobocin supplément (BS033) with 5 mL sterile distilled water or with the Novobiocin 40 mg supplement (BS056) with 20 mL of sterile water.
- Mix or vortex in order to ensure complete dissolution, avoiding the formation of foam.
- Aseptically add 0.5 mL of Novobiocin selective supplement for 100 mL of prepared base media.
- Mix well.
- Pour into sterile Petri plates and let solidify on a cold, flat surface.
- Do not invert the plates.

✓ **Reconstitution:**
31,6 g/L

✓ **Sterilization:**
Do not autoclave

✓ **Rehydration supp.:**
5 mL sterile water BS033
20 mL sterile water BS056

✓ **Add to base:**
0.5 mL / 100 mL

Use of the ready-to-melt media:

- Melt the medium (BM127) with the minimum amount of time necessary to achieve total liquefaction.
- Cool and maintain the media in a molten state at 44-47 °C.
- Pour into sterile Petri plates and let solidify on a cold, flat surface.
- Do not invert the plates.

6 INSTRUCTIONS FOR USE

- Do not dry the plates prepared above.
- Inoculate 3 drops (around 0,1 mL) of the culture of preenrichment in the center of the plate. Do not invert the plates.
- Incubate at 41,5 ± 1 °C for 24 ± 3 hours. If no migration is observed, the incubation can be prolonged another additional 24 hours.

✓ **Inoculation:**
3 drops at the center of the agar plate.

✓ **Incubation:**
24 h at 41,5 °C

NOTE
If the sample to be tested is suspected of containing a bacterial growth inhibitor, or when performing a control of disinfection/cleaning, it could be useful to add to the pre-enrichment media the appropriate disinfectant neutralizers.
If the presence of immobile *Salmonellae* are suspected, the cultures obtained from the pre-enrichment broth should be inoculated onto the appropriate media, following specific protocols.

7 RESULTS

The presence of an opaque halo centered on the point of inoculation is a presumption for *Salmonella*. Subcultures can be prepared by removing a fraction of culture from the outer edge of the halo to confirm purity and conduct additional biochemical and serological tests.

See ANNEX 1: PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media: blue powder, free-flowing and homogeneous.
Novobiocin Supplements: white pellet; giving rise after reconstitution to a colorless, limpid solution.
Prepared (complete) media: blue semi-solid agar.

Typical culture response after 24 hours of incubation at 41,5 °C (NF EN ISO 11133):

Microorganisms		Growth
Salmonella Typhimurium	WDCM 00031	whitish opaque culture with a diameter ≥ 30 mm
Salmonella Enteritidis	WDCM 00030	whitish opaque culture with a diameter ≥ 30 mm
Escherichia coli	WDCM 00013	Inhibited
Enterococcus faecalis	WDCM 00087	Inhibited

9 STORAGE / SHELF LIFE

Dehydrated media: 2-30 °C.
Freeze-dried supplements: 2-8 °C
Ready-to-melt media in vials: 2-8 °C.
The expiration dates are indicated on the labels.

Rehydrated supplement (*): 30 days at 2-8 °C
Prepared media in plates (*): 15 days at 2-8 °C, shielded from light.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated complete media
500 g bottle..... BK191HA

Dehydrated base media (without Novobiocin):
500 g bottle..... BK134HA

Novobiocin Selective Supplement:
10 x 10 mg vials BS03308
8 x 40 mg vials BS05608

Ready-to-melt media:
10 x 200 mL vials BM12708

11 BIBLIOGRAPHY

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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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ANNEX 1: PHOTO SUPPORT

MSRV MEDIUM

Detection of *Salmonella*.

Results:

Growth obtained after 24 hours of incubation at 41.5 °C.

***Salmonella* Typhimurium**

Characteristic colony:
Whitish color, presence of an opaque halo centered on the inoculation point.

Immobile *Enterobacteriaceae*

Characteristic colony:
Absence of an opaque halo; growth centered only on the inoculation point.