

# Annexe D FDIS 6579

- Need for improvement of method when used for high proportion of other enterobacteria in the sample : fecal matter or swab for the primary production environment swabs or dust.

## **D.4.3 Enrichment on selective semi-solid medium**

Modified Semi-solid Rappaport Vassiliadis (MSRV) agar plates are inoculated with the culture obtained in D.4.2.

The MSRV is incubated at  $41,5\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  for  $24\text{ h} \pm 3\text{ h}$ . If a plate is negative after 24 h it is incubated for a further  $24\text{ h} \pm 3\text{ h}$ .

# MSRV

- **Mode of Action**

De SMEDT et al. (1986) made a semi-solid RV-medium by adding agar (MSRV).

- In comparison to traditional methods this formulation gave more Salmonella-positive results.
- The detection principle is based on the motility of Salmonellae to migrate into the semi-solid medium thus forming opaque halos of growth. The motility of other microorganisms is largely inhibited by selective agents (Magnesium chloride, Malachite green and Novobiocin) and the enhanced incubation temperature of 41.5 °C.

# MSRV

## D.5.2.1 Base medium

### *Composition*

Enzymatic digest of casein	4,6	g
Acid hydrolysate of casein	4,6	g
Sodium chloride (NaCl)	7,3	g
Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ )	1,5	g
Magnesium chloride anhydrous ( $\text{MgCl}_2$ )	10,9	g
Malachite green oxalate	0,04	g
Agar	2,7	g
Water	1 000	ml

# MSRV

## *Preparation*

Aseptically add 2 ml of the novobiocin solution (D.5.2.2) to 1000 ml of base medium (D.5.2.1) at 47-50 °C. Mix carefully. = 10mg/L

The final pH should be 5,2 (5,3) – 5,4 at 20-25 °C.

Pour carefully into plates up to a final volume of 15-20 ml in Petri dishes with a diameter of 90 mm.

Allow the medium to solidify before moving and handle with care.

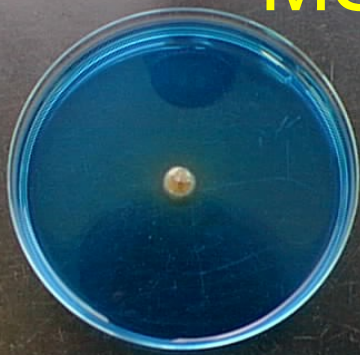
Store the plates, **with surface upwards**, for up to 2 weeks at 5 °C ± 3 °C in the dark.

**Do not invert** the plates, as the semi-solid agar is too sloppy to do so.

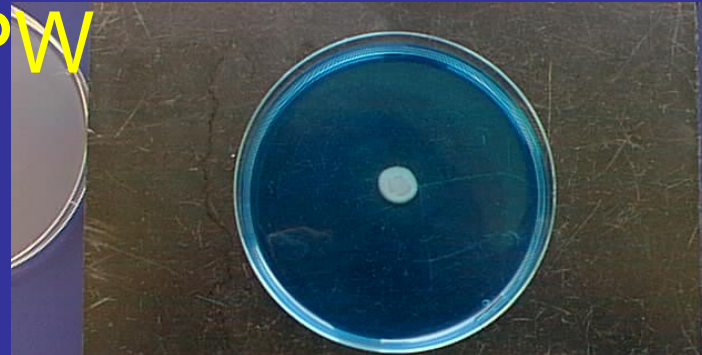
Any plates in which the semi-solid agar has liquefied or fragmented should not be used.

Immediately before use, and only if necessary, dry the surface of the agar plates carefully, for example by placing them with the lids off and the agar surface **upwards** in a Laminar Air Flow cabinet. Mind not to overdry the medium.

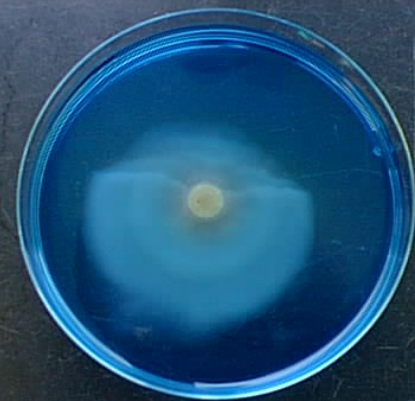
MSRV on Poultry feces pre-  
enriched in BPW



negative in 25g



positive in 25g



# MSRV on Meat

carefull it's not yet included in the scope of the annex D of ISO 6579

