

## Instructions for Use

REF - KM0150

**Rappaport-Vassiliadis (MSRV) Medium Semi-Solid**

### Dehydrated Culture Media

#### 1. Intended Use

KM0150 Rappaport-Vassiliadis (MSRV) Medium Semi-Solid is used for detecting *Salmonella* species food and environmental products.

#### 2. Composition\*

| <u>Ingredient</u>                           | <u>g/L</u> |
|---------------------------------------------|------------|
| Enzymatic digest of animal and plant tissue | 4.6        |
| Acid hydrolysate of casein                  | 4.6        |
| Sodium chloride                             | 7.3        |
| Potassium dihydrogen phosphate              | 1.5        |
| Magnesium chloride anhydrous                | 10.9       |
| Malachite green oxalate                     | 0.04       |
| Agar                                        | 2.7        |

\*Adjusted/supplemented as needed to meet performance requirements

#### 3. Summary and Explanation

A modification of Rappaport-Vassiliadis Soy Broth, the original research on MSRV Medium described by De Smedt *et al.* <sup>(1)</sup> revealed a semi-solid could be used as a rapid and sensitive test for isolating motile *Salmonella* species from food products following pre-enrichment or selective enrichment. KM0150 is formulated and tested to comply with ISO 11133:2014 <sup>(2)</sup> and ISO 6579:2017 <sup>(3,4)</sup>. *Salmonella* can be present in small numbers and is often accompanied by considerably larger numbers of other Enterobacteriaceae or bacteria of other families. Pre-enrichment is used to permit the detection of low numbers of *Salmonella* or injured *Salmonella*.

#### 4. Principle

The semi-solid medium allows motility to be detected as halos of turbid growth around the original point of inoculation. The peptones are to provide vitamins, nutrients, and nitrogen to encourage growth of *Salmonella* species. The salt maintains the osmotic balance and potassium dihydrogen phosphate is a buffer for stabilising the pH of the medium. Malachite green is included as a selective agent that inhibits Gram-positive organisms and some Gram-negative organisms.

#### 5. Preparation Instructions

Suspend 14.238g of dried culture media in 450ml of deionised/purified water and heat with frequent agitation to completely dissolve.

DO NOT AUTOCLAVE this medium.

Cool to 45-50°C and aseptically add one vial of LS0160 MSRV Novobiocin Selective Supplement (or 2ml of BM4032 MSRV Novobiocin Solution) per 450ml of product.

Gently homogenize the final medium before aseptically dispensing the specified volume into appropriate sterile containers and allowing to cool.

Prepared media may be kept at between 2 and 8°C for up to 14 days away from direct sunlight.

#### 6. Physical Characteristics

|                       | Dehydrated Medium            | Prepared Medium               |
|-----------------------|------------------------------|-------------------------------|
| Appearance and Colour | Beige/light blue fine powder | Turquoise blue semi-solid gel |
| pH                    | N/A                          | 5.1 – 5.4                     |

#### 7. Materials Provided

KM0150 can be provided in the formats detailed below. Each tub is labelled with product name, product code, lot number, expiry date, instructions, and appropriate warnings.

| Product Code      | Product Format                        |
|-------------------|---------------------------------------|
| KM0150-500G-500   | 1 x 500g Dehydrated Culture Media Tub |
| KM0150-5KG-5000   | 1 x 5kg Dehydrated Culture Media Tub  |
| KM0150-10KG-10000 | 1 x 10kg Dehydrated Culture Media Tub |

## 8. Materials Needed but not Provided

Standard microbiological laboratory materials *e.g.*, autoclave, sterile loops or swabs, collection containers, incubators, and quality control organisms.

## 9. Specimens

KM0150 Rappaport-Vassiliadis (MSRV) Medium Semi-Solid is suitable for the testing of the following specimens:

- Food and Environmental: products for human consumption, animal feed and environmental samples

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

## 10. Test Procedures and Interpretation of results

Allow the plates to come to room temperature.

### Food and Environmental Specimens <sup>(3 4)</sup>

Refer to ISO 6579:2017 <sup>(3 4)</sup> for full procedure. It is recommended that specimen is pre-enriched in a non-selective liquid medium to allow the detection of low numbers of *Salmonella* or injured *Salmonella*.

- Prepare a homogenised suspension using a ratio of 1:9 of sample to BM0082 Buffered Peptone Water (ISO) incubate according to instructions.
- After incubation, inoculate prepared KM0150 medium with 1µl of pre-enriched broth and incubate further at 41.5 ± 1°C aerobically for 24 ± 3 hours (NB: DO NOT INCUBATE INVERTED, PLATED SHOULD BE KEPT WITH THE LID UPPERMOST). It is recommended <sup>(3)</sup> that Muller Kauffmann Tetrathionate Novobiocin Broth with Iodine Complete (E&O BM0947) also be inoculated with a 1:9 ratio of pre-enriched broth to MKTTn Broth and incubated concurrently as KM0150.
- After incubation, subculture onto Xylose Lysine Deoxycholate (XLD) Agar (E&O PP0320).

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical, serological, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

## 11. Quality Control

| Organism                           | Incubation                              | Result (Specificity)                                 |
|------------------------------------|-----------------------------------------|------------------------------------------------------|
| <i>S. typhimurium</i> (NCTC 12023) | 41.5 ± 1°C aerobically for 24 ± 3 hours | Growth: turbid zone extending from inoculation point |
| <i>S. enteritidis</i> (NCTC 12694) | 41.5 ± 1°C aerobically for 24 ± 3 hours | Growth: turbid zone extending from inoculation point |
| <i>E. coli</i> (NCTC 12241)        | 41.5 ± 1°C aerobically for 24 ± 3 hours | Inhibited: possible growth without turbid zone       |
| <i>E. faecalis</i> (NCTC 12697)    | 41.5 ± 1°C aerobically for 24 ± 3 hours | Inhibited: possible growth without turbid zone       |

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium, and in agreement with any local relevant guidelines (*e.g.*, frequency, strains used, atmosphere, incubation temperature).

## 12. Performance

To fully verify KM0150 Rappaport-Vassiliadis (MSRV) Medium Semi-Solid performance, dehydrated culture media samples were used to prepare MSRV agar and tested to assess turbidity/colony morphology. Prepared samples were inoculated with 30-150cfu and incubated at 41.5 ± 1°C aerobically for 24 ± 3 hours. All samples of prepared media produced grew and showed good recovery and the correct morphology of the required test organisms: *Salmonella typhimurium* (NCTC 12023) and *Salmonella enteritidis* (NCTC 12694) and no recovery of the non-target test organisms: *Escherichia coli* (NCTC 12241) and *Enterococcus faecalis* (NCTC 12697). Therefore, it can be concluded that KM0150 Rappaport-Vassiliadis (MSRV) Medium Semi-Solid, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

## 13. Limitations of the Media

- The inhibitory agents in MSRV may inhibit certain *Salmonella*, such as *S. typhi* and *S. enterica*. Isolation techniques should include a variety of enrichment broths and isolation media.
- MSRV agar is intended for the detection of motile *Salmonella* strains and is not appropriate for the detection of non-motile *Salmonella* strains.
- Incubation for a further 24 hours can be conducted if negative results are seen on the plates.
- Biochemical and, if indicated, serological testing using pure cultures may be necessary for complete identification; these tests should be performed from a non-selective agar.
- Due to natural variation, some strains may grow poorly on this medium.

## 14. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to KM0150 Material Safety Data Sheet.

## 15. Storage conditions and Shelf life

Store product in the original container with the lid tightly closed at between 10 and 30°C in low humidity conditions away from direct sunlight. Kept under these conditions, the product may be used up to the date of expiry shown on the product label.

Do not use if the product is not free-flowing or displays any sign of colour change, formation of large lumps or hardening of the powder. Additionally, do not use medium if it has been stored inappropriately, the packaging has been damaged or has passed the expiry date.

Dehydrated culture media does not need to be used all at once; replace the cap and ensure that the container is tightly closed and stored according to labelled instructions.

Dispose of in accordance with local and national authority requirements.

## 16. References

1. De Smedt, J., Bolderdijk, R., 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(7), pp.510-514.
2. International Organization for Standardization (2014) 11133:2014 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. Geneva: ISO.
3. International Organization for Standardization (2017) 6579-1:2017 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. Geneva: ISO.
4. International Organization for Standardization (2020) 6579-1:2017/AMD 1:2020 Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of *Salmonella* spp. - Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC. Geneva: ISO.

## Version History\*

001 10/10/23 - New Document Created

\*Note: minor typographical, grammatical, and formatting changes are not included in the revision history.



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TABLE OF APPLICABLE SYMBOLS

IFU/KM0150 REV. 001

|                                                                                                                    |                                                                                                                                    |                                                                                                                         |                                                                                                                         |                                                                                                                   |
|--------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|
|  <b>REF</b><br>Catalogue number |  <b>LOT</b><br>Batch code                       |                                                                                                                         |  <b>Manufacturer</b>                 |  <b>Use by</b>               |
|  <b>Temperature limitation</b>  |  <b>Contents sufficient for &lt;n&gt; tests</b> |  <b>Consult Instructions for Use</b> |  <b>Keep away from direct light</b> |  <b>Store in a dry place</b> |