Instructions for Use

REF - KM0080 **de Man, Rogosa and Sharpe Agar (MRS)**

Dehydrated Culture Media

1. Intended Use

KM0080 de Man, Rogosa and Sharpe Agar (MRS) is used for the cultivation and enumeration of *Lactobacillus* species from a variety of sources.

2. Composition*

Ingredient	g/L
Peptone	10.0
Yeast extract	4.0
Beef extract	8.0
Glucose	20.0
Dipotassium hydrogen phosphate	2.0
Sodium acetate	4.15
Ammonium citrate	2.0
Magnesium sulphate	0.2
Manganese sulphate	0.05
Agar	10.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

This medium is predominantly used for the cultivation of *Lactobacillus* spp. from dairy and meat products. It may also be used to examine lactobacilli from humans and animals for research purposes. de Man, Rogosa and Sharpe Agar is based on the formulation by de Man, Rogosa and Sharpe [(1)]. This medium is formulated and tested in compliance with ISO 11133:2014 [(2)] and ISO 15214:1998 [(3)].

4. Principle

Peptones, beef extract, and yeast extract provide the required nitrogen and other essential nutrients. Glucose is fermented to provide energy and dipotassium hydrogen phosphate is a buffering agent to help maintain pH. Selectivity of the medium is achieved using ammonium citrate and sodium acetate, inhibiting microorganisms such as *streptococci* and moulds. The low pH also inhibits the growth of some bacteria The magnesium sulphate and manganese sulphate function as growth stimulants. Polysorbate 80 is added to the medium before sterilisation (see Section 5) and also functions as a growth supplement.

5. Preparation Instructions

Suspend 60.4g/L of dried culture media in deionised/purified water. Add 1.08 ml/L of Tween 80 and mix for 10 minutes. Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath to 45-50°C. If using the pour plate method, agar is now ready to use. If using the surface inoculation method, aseptically dispense the specified volume into appropriate sterile containers and allow to cool before inoculation.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Beige fine powder	Amber firm gel
pH	N/A	5.7 ± 0.2

7. Materials Provided

KM0080 is available 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
KM0080-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0080-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0080-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

Sufficient Polysorbate 80 for the volume of medium being prepared.

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

9. Specimens

KM0080 de Man, Rogosa and Sharpe Agar (MRS) is suitable for testing the following specimens:

- Food Industry: products for human consumption (particularly dairy products), animal feed and environmental samples.
- Colonies isolated from other media.
- Samples from humans and animals for research purposes only: faecal matter, samples from oral cavity or any other area of interest.

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

Based on sample type and information provided, determine whether the specimen requires pre-enrichment prior to inoculation of the prepared medium. If so, enrichment may be conducted in MRS broth (E&O BM4903) incubated at 37 ± 1°C in 5% CO_2 for 18-24 hours.

Inoculate plated medium directly with the sample, or subculture onto plated media after incubation in enrichment broth where required.

Clinical Specimens:

Inoculate specimen, or pre-enriched sample, directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker.

Food and Environmental Specimens:

For solid samples, prepare a homogenised suspension using a ratio of 1:10 of sample to Maximum Recovery Diluent (E&O BM0760). With homogenised and liquid specimens using the poured plate method, add 1ml to an empty Petri dish and overlay with molten but cooled medium. Rotate the dish gently to mix and allow to solidify completely. For the surface inoculation method, inoculate the agar surface with 0.1ml of sample and spread to obtain single colonies.

Specimens from Humans and Animals:

For research purposes, faecal and other human/animal samples may be added directly to the agar. Faeces may also be diluted 1:4 in sterile saline solution or 0.1% peptone water before adding 0.1ml to the agar.

Incubate at 30 ± 1°C aerobically for up to 3 days. 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)
L. brevis (NCTC 13386)	30 ± 1°C aerobically for 72 hours	Growth: Cream colonies
L. lactis (NCTC 6681)	30 ± 1°C aerobically for 72 hours	Growth: Cream colonies
L. sakei (NCTC 13842)	30 ± 1°C aerobically for 72 hours	Growth: Cream colonies
E. coli (NCTC 12241)	30 ± 1°C aerobically for 72 hours	Inhibited
B. cereus (NCTC 10320)	30 ± 1°C aerobically for 72 hours	Inhibited

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 KM0080 de Man, Rogosa and Sharpe Agar (MRS) performance, dehydrated culture medium samples were used to prepare de Man, Rogosa and Sharpe Agar and tested to assess colony morphology and recovery level (where an acceptable range is ≥70% and ≤120%) compared to a non-selective reference medium. Prepared samples were inoculated with 30-150cfu for the target organisms and 10^4-10^5 cfu for the non-target organisms then incubated at 30 ± 1°C aerobically for 72 hours. All samples of prepared media showed good recovery and the correct morphology of the required test organisms: *Lactobacillus brevis* (NCTC 13386), *Lactococcus lactis* (NCTC 6681) and *Lactobacillus sakei* (NCTC 13842) and no recovery of the non-target test organisms: *Escherichia coli* (NCTC 12241) and *Bacillus cereus* (NCTC 10320). Therefore, it can be concluded that KM0080 de Man, Rogosa and Sharpe Agar (MRS), meets performance criteria when used according to the instructions outlined above.

13. Limitations of the Medium

- Due to natural variation, some strains may grow poorly on this medium.
- Some growth of *Leuconostoc* spp. and pediococci may occur.
- Sterilisation of this medium at 121°C for 15 minutes, may cause the pH to fall outside specified pH limits. If this occurs, adjust the pH of the medium before sterilisation using acetic acid or sodium hydroxide to achieve required pH after sterilisation.
- Biochemical, serological, molecular, or mass spectrometry testing using pure cultures may be necessary for complete identification.

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 KM0080 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 medium 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 Man, J., Rogosa, M., and Sharpe, M. (1960) A Medium for the cultivation of Lactobacilli. J. Appl. Bact., 23, pp.130-135.
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 (1998) 15214:1998 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of mesophilic lactic acid bacteria - Colony-count technique at 30 degrees C. Geneva: ISO.

Version History*

001 25/09/23 - New Document Created

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

EC REP

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TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code		Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place