Instructions for Use

REF - KM0159 **SIM Medium**

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use. KM0159 SIM Medium is a multi-purpose medium for the differentiation of *Enterobacteriaceae* and *Clostridium* spp.

2. Composition*

Ingredient	g/L
Enzymatic digest of casein	20.0
Enzymatic digest of animal tissue	6.1
Ferric ammonium citrate	0.2
Sodium thiosulphate	0.2
Agar	3.5

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

SIM Medium allows for the evaluation of hydrogen sulphide production, indole formation, and motility. These characteristics aid in identifying *Enterobacteriaceae*, particularly *Salmonella* and *Shigella* spp. SIM Medium has now been included in ISO 15213-2:2023 [(1)] as a confirmation medium for *Clostridium perfringens*.

4. Principle

The presence of motility is apparent by the organism tracking out from the line of inoculation and often turning the medium turbid. Non-motile organisms generally grow within the stab line leaving the surrounding medium clear. H_2S positive organisms turn the medium black due to the formation of hydrogen sulphide in the presence of the sodium thiosulfate and ferric ammonium citrate often making it difficult to determine the other parameters.

Indole production is tested for by layering a small amount of Indole Reagent (Kovac's) onto the surface of the medium. A positive result is indicated by the formation of a pink colour at the interface of the reagent and the medium. The enzymatic digest of casein and enzymatic digest of animal tissue provide the required carbon, nitrogen, and vitamins in this medium. Ferric ammonium citrate and sodium thiosulfate are used to detect hydrogen sulphide production. The low concentration of agar allows for a semisolid media which is used for motility detection.

5. Preparation Instructions

Suspend 30.0g/L of dehydrated culture medium in 1 litre of deionised/purified water and allow to soak whilst mixing for 10 minutes.

Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath to 45-50°C.

Aseptically dispense the specified volume into appropriate sterile containers and allow to cool.

Prepared media may be kept at between 2 and 8°C for up to 6 months away from direct sunlight.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Straw fine powder	Dark straw semi-solid gel
pH	N/A	7.3 ± 0.2

7. Materials Provided

KM0159 SIM Medium 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
KM0159-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0159-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0159-10KG-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, media for isolation and quality control organisms.

9. Specimens

KM0159 SIM Medium is suitable for the testing of the following specimens:

- Specimens should be isolated colonies or pure cultures.

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 media to come to room temperature.

Isolated colonies or stock cultures:

Inoculate the media by stabbing nearly to the bottom with a wire loop or equivalent using a pure culture (or discrete single colony) of the test organism. Incubate at 35 ± 2°C aerobically for 18-24 hours. Incubate at 37 ± 1°C anaerobically for 18-24 hours if confirming the presence of *Clostridium* species.

Following incubation, examine the media to determine whether the organism is motile. Motility is shown by the organism tracking out from the line of inoculation and often turning the medium turbid. Non-motile organisms generally grow within the stab line leaving the surrounding medium clear.

H_2S positive organisms (e.g., *Proteus* spp.) turn the medium black due to the formation of hydrogen sulphide; this can often make it difficult to determine the other parameters.

Indole production is tested for by layering a small amount of Indole Reagent onto the surface of the medium. A positive result is indicated by the formation of a purple colour at the interface of the reagent and the medium.

After incubation, examine media for morphology (typical results outlined in Quality Control table below). Perform further biochemical or serological testing as required to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
S. flexneri (ATCC 12022)	37 ± 1°C aerobically for 18-24 hours	Motility = -ve. Indole = -ve. H_2S production = -ve
E. coli (NCTC 12241)	37 ± 1°C anaerobically for 18-24 hours	Motility = +ve. Indole = +ve. H_2S production = -ve
C. perfringens (NCTC 8237)	37 ± 1°C anaerobically for 18-24 hours	Motility = -ve, Indole = -ve, H_2S production = +ve
S. typhimurium (NCTC 12023)	37 ± 1°C aerobically for 18-24 hours	Motility = +ve. Indole = -ve. H_2S production = +ve
S. sonnei (NCTC 8574)	37 ± 1°C aerobically for 18-24 hours	Motility = -ve. Indole = -ve. H_2S production = -ve
S. enteritidis (NCTC 12694)	37 ± 1°C aerobically for 18-24 hours	Motility = +ve. Indole = -ve. H_2S production = +ve

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 KM0159 SIM Medium performance, dehydrated culture media samples were used to prepare SIM Medium and tested to assess colour change, gas production, and indole presence when incubated at 37 ± 1°C for 18-24 hours in the atmosphere indicated in the Quality Control table. All samples of prepared media produced grew and showed good recovery and the correct morphology of the required test organisms: *Shigella flexneri* (ATCC 12022), *Escherichia coli* (NCTC 12241), *C. perfringens* (NCTC 8237), *Salmonella typhimurium* (NCTC 12023), *Shigella sonnei* (NCTC 8574) and *Salmonella enteritidis* (NCTC 12694). Therefore, it can be concluded that KM0159 SIM Medium, meets performance criteria when used according to the instructions outlined above.

13. Limitations of the Media

- Due to natural variation, some strains may grow poorly on this medium.
- Inoculation of SIM Medium from a liquid or broth suspension may result in a delay in the initiation of growth.

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 KM0159 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. International Organization for Standardization (2023) 15213-2:2023 Microbiology of the food chain – Horizontal method for the detection and enumeration of Clostridium spp. Geneva, ISO.

Version History*

001 10/10/23 - New Document Created
002 22/05/24 – Update to shelf-life and QC Table
003 08/08/24 – Inclusion of ISO 15213-2:2023

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

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TABLE OF APPLICABLE SYMBOLS

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