Instructions For Use
ENGLISH

SIM Medium

Semisolid medium for the identification of Enterobacteriaceae by sulphide, indole and motility tests.

DESCRIPTION

SIM Medium is used for the identification of microorganisms from clinical specimens and other samples on the basis of hydrogen sulphide production, indole formation, and motility.

This medium is an aid to identify and discriminate between species of the family Enterobacteriaceae - for example, *Enterobacter* has the general characteristics of *Klebsiella* species but can be differentiated because they are motile as well as *Citrobacter*, *Proteus*, *Providencia* and *Serratia; Klebsiella, Enterobacter, Hafnia* and *Serratia* species are usually indole negative whereas *Escherichia* species are positive for indole (except *E. vulneris*); unlike *Shigella*, *Salmonella* species possess flagella and hence are motile and most produce hydrogen sulphide (except *S.* Paratyphi A and *S.* Typhi, which is a weak producer).

TYPICAL FORMULA*	(g/litre)
Casein Peptone	20.0
Meat Peptone	6.1
Ferric Ammonium Sulphate	0.2
Sodium Thiosulphate	0.2
Agar	3.5
Final pH 7.3 ± 0.2 at 25°C	

*Adjusted and/or supplemented as required to meet performance criteria.

METHOD PRINCIPLE

Peptones provide carbon, nitrogen and amino acids for bacterial growth. Casein peptone in particular contains tryptophan which is converted to indole. Ferric ammonium sulphate and sodium thiosulphate are used to detect hydrogen sulphide (H_2S) production through formation of a black precipitate. Agar is the solidifying agent. The low concentration of agar makes the medium semisolid allowing for visual determination of motility.

PREPARATION

Dehydrated medium	Suspend 30.0 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil and shake until completely dissolved. Dispense into tubes. Sterilize in autoclave at 121°C for 15 minutes.
Medium in bottles	Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into tubes.

Note: Sufficient volume of medium must be dispensed into tubes to give a depth of about 4 cm. Allow tubes to solidify in a vertical position.

TEST PROCEDURE

1. Inoculate test organism by stabbing two-thirds into the medium.
2. Incubate with loose caps at 35 ± 2°C for 18-24 hours.
3. After incubation, examine tubes for motility and H_2S production.
4. Once H_2S and motility have been recorded, add 3-4 drops of Kovac's Reagent (ref. 87001) to each tube.

Note: Test organisms must be in pure culture. The inoculum should be taken from a solid medium as inoculum from liquid suspensions may delay results. Erroneous results may occur if caps are not loose during incubation.

INTERPRETING RESULTS

Motility	A positive motility test is indicated by a diffuse growth/turbidity extending from inoculating stab line, whereas non-motile organisms grow only along the line of inoculation.
H_2S	Hydrogen sulphide production is shown by a blackening of the medium in those areas where microbial growth has occurred.
Indole	Indole formation is seen as appearance of a pink or red color, whereas test is negative if there is no color change after addition of Kovac's Reagent.

Consult appropriate references for activities of specific microorganisms and for complete identification of Enterobacteriaceae[1-4].

Examples of characteristic reactions.

Microorganisms	Sulphide	Indole	Motility
Citrobacter	+	+	+
Enterobacter	–	–	+
Escherichia	–	+	±
Hafnia	–	–	+
Klebsiella	–	+	–
Morganella	–	+	+
Proteus mirabilis	+	–	+
Proteus vulgaris	+	+	+
Providencia	–	+	+
Salmonella	+	–	+
Serratia	–	–	+
Shigella	–	±	–
Yersinia enterocolitica	–	±	±*

**Y. enterocolitica* are motile at room temperature (25°C) but non-motile at 37°C.

APPEARANCE OF THE MEDIUM

Dehydrated medium: free-flowing, homogeneous, beige.
Prepared medium: semisolid, clear to slightly opalescent, medium amber.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store tubes and bottles at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Bottles: 2 years.
Tubes: 1 year.

QUALITY CONTROL

To check the performance of the medium the following microbial strains can be used.

Strain		Inoculum	Incubation	Growth	H_2S	Indole	Motility
Escherichia coli	ATCC® 25922	2-3 colonies; direct inoculum	35 ± 2°C / 18-24 h	Good	–	+	+
Salmonella Typhimurium	ATCC® 14028			Good	+	–	+
Shigella flexneri	ATCC® 12022			Good	–	–	–

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for *in vitro* diagnostic use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. UK Standards for Microbiology Investigations ID 16: Identification of Enterobacteriaceae (2015). Issued by the Standards Unit, Microbiology Services, PHE. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/423601/ID_16i4.pdf
2. UK Standards for Microbiology Investigations ID 24: Identification of *Salmonella* species (2015). Issued by Standards Unit, Microbiology Services, PHE. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/443443/ID_24i3.pdf
3. UK Standards for Microbiology Investigations ID 20: Identification of *Shigella* species (2015). Issued by Standards Unit, Microbiology Services, PHE. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/423180/ID_20i3.pdf
4. UK Standards for Microbiology Investigations ID 21: Identification of *Yersinia* species (2015). Issued by Standards Unit, Microbiology Services, PHE. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/443392/ID_21i3.pdf
5. Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (1995) Manual of clinical microbiology. 6th ed. American Society for Microbiology, Washington, D.C.
6. American Public Health Association: Compendium of methods for the microbiological examination of foods. - 3rd ed. (1992).
7. Tittsler, R. P. and L. A. Sandholzer (1936) The use of semi-solid agar for the detection of bacteria motility. J. Bact. 31:575.

TABLE OF SYMBOLS

IVD *In vitro* Medical Diagnostic Device	Manufacturer	Temperature limitation	Do not reuse
LOT Batch code	Use by	Contains sufficient for <n> tests	Keep away from sunlight
REF Catalogue number	Fragile, handle with care	Consult Instruction For Use	

The product is available in the various configurations listed below. There may be additional product ref. numbers as well. For an updated listing of available products, visit **liofilchem.com**

Product	Format	Packaging	Ref.
SIM Medium	Tube	20 x 9 ml	24479
SIM Medium	Tube	100 x 10 ml	26095
SIM Medium	Bottle	6 x 100 ml	403050
SIM Medium	Dehydrated medium	500 g of powder	610181
SIM Medium	Dehydrated medium	100 g of powder	620181

This document is available from the online Support Center:
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