SIM AGAR ISO

Dehydrated and ready-to use culture medium

1 - INTENDED USE

Semisolid medium for the confirmation test of *Clostridium perfringens* according to ISO 15213-2.

2 - COMPOSITION - TYPICAL FORMULA* (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	6.0 g
Enzymatic digest of soya	20.0 g
Ferrous ammonium sulphate (anhydrous)	0.2 g
Sodium thiosulphate	0.2 g
Agar	3.6 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

SIM Agar ISO is prepared according to the formulation described in ISO 15213-2, for the confirmation test of *C. perfringens* colonies, isolated from the food chain.[1]
Compared to the classical medium for the differentiation of *Enterobacteriaceae*[2] (SIM Bios Medium REF 402036), the formulation described by ISO 15213-2[2] contains soy peptone instead of casein peptone and ferrous ammonium sulphate instead of ferric ammonium citrate.
Peptones provide carbon, nitrogen and trace elements for bacterial growth. Ferrous ammonium sulphate is as an indicator of the formation of hydrogen sulphide. H_2S positive strains produce thiosulphate reductase that cause the release of a sulfide molecule from sodium thiosulfate present in the medium; this sulfide molecule couples with a hydrogen ion to form H_2S gas that reacts with the ferrous ammonium sulphate, forming ferrous sulphide, resulting in a black precipitate.
Peptone is rich in tryptophan, that is hydrolysed by tryptophanase to produce three possible end products: indole, pyruvate and ammonia. Indole production is detected by Kovacs' reagent, which reacts with indole to produce a red-coloured compound.
The detection of bacterial motility is favoured by the low concentration of agar: in the semi-solid medium, motile bacteria 'swarm' and give a diffuse spreading growth that is easily recognized by the naked eye.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Fill 10 ml into tubes and sterilize by autoclaving at 121 °C for 15 min. Allow to cool in upright position.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	pale yellow, clear or slightly opalescent
Final pH at 20-25 °C	7.3 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
SIM Agar ISO	Dehydrated medium	4020372	500 g (16.6 L)
	Ready-to-use tubes	552037	20 x 10 mL glass tubes, 17x125 mm, flat bottom, aluminium screw-cap. Packaging: cardboard box

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave and water-bath, sterile needles, incubator and laboratory equipment as required, ancillary culture media and reagents for the complete identification of the culture (Kovacs Reagent REF 19171000).

8 - SPECIMENS

SIM Agar ISO is not intended for primary isolation from specimens; it is inoculated with pure colonies from a culture on solid media.

9 - TEST PROCEDURE

Colonies grown on TSC Agar and sub-cultured anaerobically on blood agar plates or nutrient-rich rich plates are stabbed into SIM Agar tubes for the confirmation test of *C. perfringens*.
Incubate the tubes anaerobically with the caps loosened for 22 h ± 2 h at 37 °C ± 1 °C.

10 - READING AND INTERPRETATION

After incubation the tubes are read for H_2S production, motility test, indole production
Determine motility and hydrogen sulfide production before the addition of the reagent for determination of indole production.
H_2S production: blackening along stab line or extensive blackening of medium; negative test: no blackening
Motility positive: diffuse growth outside the inoculation stab line; a negative motility test is indicated by growth confined to the stab line.
Indole production: add 3-4 drops of Kovacs' reagent; positive test: tubes giving a red coloured ring; negative test: tubes giving a yellow-coloured ring.
C. perfringens is positive for hydrogen sulfide production and negative for indole production and motility.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in

compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	H_2S	MOTILITY	INDOLE
C. perfringens ATCC 13124	36-38°C / 20-24H /AN	+	-	-
E. coli ATCC 25922	36-38°C / 20-24H /AN	-	+	+

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated and ready to use SIM Agar is tested for performances characteristics comparing the results with a previously approved Reference Batch.
Pure colonies cultivated on blood agar plates of the following strains are inoculated by stabbing the medium in tubes: *E. coli* ATCC 25922, *C. perfringens* ATCC 13124, C. perfringens ATCC 12916, C. *perfringens* NCTC 13170. After incubation at 37°C for 20-24 hours anaerobically, motility, H_2S and indole production are observed and recorded. All strains show performances characteristics according to the specifications.

13 - LIMITATIONS OF THE METHOD

- Do not take inoculums from liquid or broth suspension.
- It is necessary to inoculate the medium taking care to remove the needle along the same stabbing line.
- Hydrogen sulfide reactions are intensified by motile cultures.[1]
- Motile bacteria but with damaged flagella can give false negative results.

14 - PRECAUTIONS AND WARNINGS

- The products are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- Apply Good Manufacturing Practice in the production process of prepared media.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each tube is for single use only.
- Ready-to-use tubes of SIM Agar ISO are subject to terminal sterilization by autoclaving.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized media inoculated with samples or microbial
- strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the shelf life of the finished products, according to the type (plates/tubes/bottles) and the storage method (temperature and packaging).

Ready-to-use tubes

Upon receipt, store tubes in their original pack at +2/+8°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. Before use, check the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g. microbial contamination, atypical colour).

16 - REFERENCES

1. ISO 15213-2:2023. Microbiology of the food chain - Horizontal method for the detection and enumeration of Clostridium spp. Part 2: Enumeration of sulfite-reducing Clostridium spp. by colony-count technique.
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 0	First issue	2024/03

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

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