SIM Agar ISO 15213-2

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

INTENDED PURPOSE

Confirmation medium for enumeration of *Clostridium perfringens,* in food, feed, environmental samples and samples from the primary production stage. This medium is not intended for use in the diagnosis of disease or other conditions in humans.

DESCRIPTION

Clostridium perfringens is a gram-positive, anaerobic, spore-forming bacterium. As a ubiquitous bacterium, *C. perfringens* is predominantly found in soil, but also in the intestinal tract of humans and animals. Therefore, the presence of *C. perfringens* in high numbers can be an indication of inadequate preparation or handling of food.

SIM (Sulfite Indole Motility) Agar allows the differentiation of microorganisms and identification of *C. perfringens* on the basis of sulfite production, indole formation, and motility.

It complies with the specification given by ISO 15213-2.

TYPICAL FORMULA* (Per Litre of Purified Water)

Enzymatic Digest of Casein	6.0 g
Enzymatic Digest of Soya	20.0 g
Ferrous Ammonium Sulfate, anhydrous ($FeH_8N_2O_8S_2$)	0.2 g
Sodium Thiosulfate ($Na_2O_3S_2$)	0.2 g
Agar	3.5 g
Final pH 7.0 ± 0.2 at 25°C	

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

METHOD PRINCIPLE

Peptone and enzymatic digest of casein provide amino acids, nitrogen, carbon, vitamins and minerals required for organisms growth. Ferrous ammonium sulfate and sodium thiosulfate serve as indicators for hydrogen sulfide (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

Suspend 29.9 g of the powder in 1 liter of distilled or deionized water. Heat shaking frequently until completely dissolved. Pour 10 ml into tubes. Autoclave at 121°C for 15 minutes. Allow to cool in upright position.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as: Autoclave, Test tubes, inoculating loops, incubator, quality control organisms.

TEST PROCEDURE

Following the procedure outlined in ISO 15213-2, stab a SIM agar tube with colonies grown anaerobically on blood agar plates or nutrient agar plates. Incubate at 37 ± 1°C for 20-24 hours in an anaerobic atmosphere with loosen caps.

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.

For more details, consult appropriate guidance.

INTERPRETING RESULTS

After incubation the tubes are examined for:

- sulfite production: tubes showing blackening are positive;
- motility: tubes showing growth outside the inoculation stab are positive;
- indole production: tubes giving a red colored ring directly after adding Kovacs reagent are positive.

C. perfringens is positive for sulfite production and negative for indole production and motility.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. 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
Medium in tubes: 2 years

QUALITY CONTROL

Appearance of Dehydrated Medium: Free-flowing, homogeneous, beige.

Appearance of Prepared Medium: Semisolid, clear to slightly opalescent, medium amber.

Expected Cultural Response:

Control strain	Incubation	Criteria	Specification
Clostridium perfringens WDCM 00007 (ATCC® 13124; NCTC 8237)	22 ± 2 h / 37 ± 1 °C / anaerobic atmosphere	Good growth	Positive reaction: Blackening of the tube, no growth outside the inoculation stab and no red colored ring after adding Kovacs reagent
Escherichia coli WDCM 00013 (ATCC® 25922; NCTC 12241)		Good growth	Negative reaction: No blackening of the tube, possible growth outside the inoculation stab and red coloured ring after adding Kovacs reagent

Please refer to the actual batch related Certificate of Analysis (CoA).

PERFORMANCE CHARACTERISTICS

Performance testing of SIM Agar ISO 15213-2 was carried out using the QC strains listed above. The results obtained met the established criteria.

LIMITATIONS

Invalid results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes.

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

WARNING AND PRECAUTIONS

For professional use only. Operators must be trained and have certain experience. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

DISPOSAL OF WASTE

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

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

ORDER INFORMATION

Product	Format	Packaging	Ref.
SIM Agar ISO 15213-2	Tube	20 x 10 ml	24132
	Dehydrated medium	100 g	620062
		500 g	610062

Revision History

Revision	Release Date	Change Summary
0	2024-08-22	Document creation
1	2024-11-27	Added new packaging

This IFU document and the SDS are available from the online Support Center:

liofilchem.com/ifu-sds

References

1. EN ISO 15213-2:2023. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Clostridium* spp. — Part 2: Enumeration of *Clostridium perfringens* by colony-count technique.
2. EN ISO 11133:2014+Amd1:2018+Amd2:2020. Microbiology of food, animal feed and water -- Preparation, production, storage and performance testing of culture media.
3. Tittsler, R. P. and L. A. Sandholzer 1936. The use of semi-solid agar for the detection of bacteria motility. J. Bact. 31:575.
4. 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.
5. American Public Health Association: Compendium of methods for the microbiological examination of foods. - 3rd ed. 1992.

Table of Symbols

LOT	Batch code
REF	Catalogue number
	Manufacturer
	Use by
	Fragile, handle with care
	Temperature limitation
	Contains sufficient for <n> tests
	Consult instructions for use
	Do not reuse
	Keep away from sunlight

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