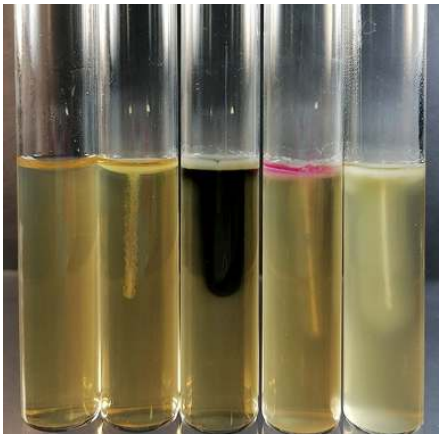
INSTRUCTIONS FOR USE

SIM BIOS MEDIUM

Dehydrated culture medium



SIM Bios Medium: from left: uninoculated tube, *K.pneumoniae* (not motile), *S.arizonae* H_2S+, *E.coli* (motile, indole+), *E.aerogenes* (motile)

1 - INTENDED USE

In vitro diagnostic. Differential medium used as an aid in identifying Gram negative *Enterobacteriaceae,* especially *Salmonella* and *Shigella*, by ability to produce indole and hydrogen sulphide and exhibit motility.

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

Tryptone	20.0 g
Peptone	6.1 g
Ferric ammonium citrate	0.2 g
Sodium thiosulphate	0.2 g
Agar	3.5 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 (Sulfide Indole Motility) Bios Medium is used for the differentiation of *Enterobacteriaceae*, especially *Salmonella* and *Shigella*, based on the production of H_2S, indole and the mobility test.[1,2]

Peptones provide carbon, nitrogen and trace elements for bacterial growth. Ferric ammonium citrate 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 ferric ammonium citrate, forming ferrous sulphide, resulting in a black precipitate. Tryptone is rich in tryptophan, that is hydrolysed by tryptophanase to produce three possible end products indole, pyruvate and ammonia. Indole production is detected by Kovac's reagent which contains 4 (p)-dimethylamino benzaldehyde: this 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.

The medium does not contain carbohydrates since they are inhibitory for tryptophanase[3] and for the production of iron sulphide[4].

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, distribute in tubes and sterilize by autoclaving at 121°C for 15 minutes. Cool in upright position.

5 - PHYSICAL CHARACTERISTICS

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

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
SIM Bios Medium	Dehydrated medium	4020362	500 g (16.6 L)

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 Bios Medium is not intended for primary isolation from clinical specimens; it is inoculated with pure colonies from a culture on solid media, isolated from clinical specimens or other materials.

9 - TEST PROCEDURE

With inoculating needle stab two-thirds the depth of medium in the centre of tube. Incubate tubes with loosened caps, aerobically, at 35-37°C for 18-24 hours.

10 - READING AND INTERPRETATION

Determine motility and H_2S production before the addition of the reagent for determination of indole production.

Motility positive: diffuse growth outward away from stab line or turbidity of the medium; a negative motility test is indicated by growth confined to the stab line.

H_2S production: blackening along stab line or extensive blackening of medium; negative test: no blackening

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Indole production: add 3-4 drops of Kovacs' reagent. Positive test: red colour in upper position of medium; negative test: yellow colour in upper position of medium.

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
E.coli ATCC 25922	35-37°C / 18-24H /A	-	+	+
*S.*Typhimurium ATCC 14028	35-37°C / 18-24H /A	+	+	-
S.sonnei ATCC 9290	35-37°C / 18-24H /A	-	-	-

A: aerobic 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 SIM Bios Medium is tested for performances characteristics comparing the results with a previously approved Reference Batch.
Pure colonies cultivated on Tryptic Soy Agar of 6 Gram negative strains are inoculated by stabbing the medium in tubes: *E.coli* ATCC 25922, *S.sonnei* ATCC 9290, *K.pneumoniae* ATCC 23357, *C.freundii* ATCC 8090, *P.vulgaris* ATCC 9484, *S.*Typhimurium ATCC 14028. After incubation at 35-37°C for 18-24 hours aerobically, motility, H_2S and indole production are observed and recorded. All strains show performances characteristics according to the specifications for both batches.

13 - LIMITATIONS OF THE METHOD

- The tests that can be performed with the SIM Bios Medium are not sufficient to identify *Enterobacteriaceae* at the species level.
- 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]
- Studies of Edmondson and Sanford showed that non-motile mucoid *Klebsiella* strains may give false positive motility reaction; this is due to mucoid strains spilling between medium and tube giving a cloudy appearance which is often confused with motility.[5]
- Temperature-dependent mobility is observed for many strains of *Yersinia enterocolitica*; the ambiguous results that are reported for this microorganism do not recommend adopting the mobility test in the identification schemes for *Y. enterocolitica*.[6]
- Motile bacteria but with damaged flagella can give false negative results.
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification.
- This culture medium is intended as an aid in the diagnosis of infectious disease; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is 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.
- 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.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- 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

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).

16 - REFERENCES

1. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
2. Atlas D, Snyder J. Media Reagents and Stains. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.345.
3. Freundlich M, Lichstein HC. Inhibitory effect of glucose on thryptophanase. J Bacteriol 1960; 80:633-638.
4. Bulmash JM, Fulton M. Discrepant tests for hydrogen sulfide J. Bact 1964;88:1813.
5. Edmondson EB, Sanford JP. The Klebsiella-Enterobacter (Aerobacter)-Serratia group. Medicine 1967; 46(4): 323.
6. D'Amato RF, Tomfohrde KM. Influence of Media on Temperature-Dependent Motility Test for Yersinia enterocolitica. J Clin Microbiol 1981; 14:347-348.

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

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/05
Revision 2	Update of "precautions and warnings" and "storage conditions and shelf life"	2022/03
Revision 3	Removal of obsolete classification	2023/04

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

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