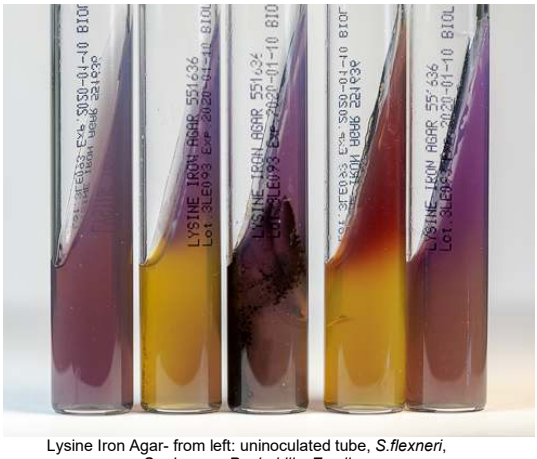
INSTRUCTIONS FOR USE

LYSINE IRON AGAR

Dehydrated culture medium



Lysine Iron Agar- from left: uninoculated tube, *S.flexneri*, *S.arizonae*, *P.mirabilis*, *E.coli*

1 - INTENDED USE

In vitro diagnostic. For the differentiation of some members of *Enterobacteriaceae*, especially *Salmonella*, isolated from clinical and non-clinical specimens.

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

Peptone	5.00 g
Yeast Extract	3.00 g
Glucose	1.00 g
L-Lysine	10.00 g
Fe-Ammonium Citrate	0.50 g
Sodium Thiosulphate	0.04 g
Bromocresol Purple	0.02 g
Agar	15.00 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

Edwards and Fife[1] in 1961 devised a medium to solve the problem of misidentification of strong lactose-fermenters strains of *Arizona (*now *Salmonella enterica* subsp. *arizonae*) that didn't produce blackening of TSI or KIA tubes.
Johnson et al.[2] in 1965 described a method based on primary differentiation of various groups of bacteria by the use of Kligler iron agar and lysine iron agar, and triple sugar iron agar and lysine iron agar for the identification of *Salmonella*, *Shigella*, and *Arizona* group isolated from stool.
Lysine Iron Agar (LIA), prepared according to the formula proposed by Edwards and Fife[1], aids in the differentiation of some members of rapid lactose-fermenter *Enterobacteriaceae*, especially *S.arizonae*, isolated from clinical and non-clinical specimens, by means of deamination or decarboxylation of lysine and production of hydrogen sulphide.[3] The medium is included in the FDA-BAM[4] schemes for the identification of *Salmonella* from food, together with other biochemical tests.
Lysine iron agar contains lysine, peptones, a small amount of glucose, a pH indicator, ferric ammonium citrate, and sodium thiosulfate.
Peptone and yeast extract provide nitrogen, carbon, vitamins and trace elements for bacterial growth. Glucose is the fermentable carbohydrate. Bromocresol purple is a pH indicator that changes to a yellow colour at or below pH 5.2 and is purple at or above 6.8. Sodium thiosulfate and ferric ammonium citrate allow for hydrogen sulphide detection: strains that produce hydrogen sulphide cause blackening of the medium due to ferrous sulphide production. Lysine is included for the detection of decarboxylase and deaminase enzymes.
Lysine decarboxylation is an anaerobic process which occurs in the butt of the medium; lysine deamination is an aerobic process which occurs on the slant.
Lysine decarboxylase removes the COOH group from lysine to produce CO_2 and cadaverine, an alkaline polyamine which neutralizes the organic acids formed by glucose fermentation, and the butt of the medium reverts to the alkaline state (purple). If the decarboxylase is not produced, the butt remains acidic (yellow). If oxidative deamination of lysine occurs, α-ketocarboxylic acid is formed that reacts with ferric ions near surface of medium under influence of oxygen, to form a reddish-orange compound; the combination of this compound with bromocresol purple produces a distinct red colour on the slant. If deamination does not occur, the slant remains purple.[3]
Within *Enterobacteriaceae*, *Salmonella*, with the sole exception of *S.*Paratyphi A, is the only genus that rapidly decarboxylates lysine and produces hydrogen sulphide: on Lysine Iron Agar these two characteristics are clearly visible both for the lactose-fermenting strains and for the lactose non-fermenting strains.
Deamination of lysine is a characteristic of *Proteus*, *Providencia* and *M.morganii*, the only members of *Enterobacteriaceae* that produce lysine deaminase enzyme.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 34.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Distribute in tubes, and sterilize by autoclaving at 121°C for 15 minutes. Cool in slanted position to obtain a deep butt.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey-purple, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	purple, limpid
Final pH at 20-25 °C	6.7 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Lysine Iron Agar	Dehydrated medium	4016362	500 g (14,5 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile microbiological needles, Erlenmeyer flasks, screw capped tubes, incubator and laboratory equipment as required, ancillary culture media and reagents for complete identification of the colonies.

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8 - SPECIMENS

The specimens consist of bacteria strains isolated from clinical specimens or other samples, purified on appropriate medium (e.g. Tryptic Soy Agar or Blood Agar).

9 - TEST PROCEDURE

With a straight inoculating needle, inoculate by stabbing through the centre of the medium to the bottom of the tube and then streaking the slant.
Incubate the tubes aerobically, with the loosened caps so that aerobic conditions prevail on the slant, at 35 ± 2°C for 24 ± 2 hours.
Unpublished data have demonstrated that 48 hours reading of LIA slants has no diagnostic value.[4]

10 - READING AND INTERPRETATION

After incubation, observe the colour changes in the butt and on the slant.
Lysine decarboxylation (detected in the butt):
Positive test: purple slant/purple butt (alkaline), the butt reaction may be masked by H_2S production.
Negative test: purple slant/yellow butt (acid), fermentation of glucose only.
Lysine deamination (detected on the slant):
Positive test: red slant
Negative test: slant remains purple
H_2S production:
Positive test: black precipitate
Negative test: absence of black precipitate

Typical reactions by members of the *Enterobacteriaceae*

Organism	Slant	Butt	H_2S
Salmonella	AK	AK	+
S.arizonae	AK	AK/N	+
*S.*Paratyphi A	AK	A	-
Shigella	AK	A	-
Escherichia	AK	A/N	-
Klebsiella	AK/N	AK/N	-
Citrobacter	AK	A	+ or -
Proteus	R	A	-
Providencia	R	A	-

AK: alkaline reaction, purple colour; A: acid reaction, yellow colour; R: red colour (lysine deamination); N: neutral reaction, no colour change; +: positive reaction; -: negative reaction

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.

*S.*Typhimurium ATCC 14028	growth, purple slant, purple butt, H_2S +
Proteus mirabilis ATCC 12453	growth, red slant, yellow butt, H_2S -
S.flexneri ATCC 12022	growth, purple slant, yellow butt, H_2S -

Aerobic incubation at 35± 2°C for 18-24 hours.
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 Lysine Iron Agar is tested for specific performance characteristics by comparing the results with a previously approved Reference Batch. Pure cultures, grown for 18–24 h on Tryptic Soy Agar, of the following strains are inoculated directly into the tubes by stabbing the butt and streaking the slant: *P.vulgaris* ATCC 9484, *P.mirabilis* ATCC 12453, *P.rettgeri* ATCC 39944, *S.*Typhimurium ATCC 14028, *S.arizonae* clinical isolate, *S.flexneri* ATCC 12022, *E.coli* ATCC 25922, *C.freundii* ATCC 8090. The tubes are incubated with loosened caps at 35–37 °C for 18-24 hours. The colour changes of medium on the slant and in the butt are observed and recorded: for all strains the reactions are conform to the specifications.

13 - LIMITATIONS OF THE METHOD

- It is necessary to inoculate the medium with a microbiological needle without breaking the agar (do not use loops).
- H_2S producing *Proteus* spp. do not blacken LIA.[3]
- Ferrous sulphide may not be seen with organisms that do not decarboxylate lysine because acid in the butt may suppress its formation; for this reason and for distinguishing coliforms from Shigella, it is recommended to use LIA in conjunction with TSI or KIA media.[3]
- Red slant reaction with *M.morganii* may be variable after 24 hours of incubation; complete deamination of lysine usually requires longer incubation (up to 48 hours).[3]
- On Lysine Iron Agar, gas production is normally irregular or suppressed, with the sole exception of *Citrobacter*.[3]
- *Salmonella enterica* ser. Paratyphi A does not decarboxylate lysine and the reactions are: K / A, H_2S -.
- Lysine Iron Agar is not a substitute for the lysine decarboxylation test on Moeller Decarboxylase Medium.
- The lysine decarboxylation/deamination is one of the tests necessary for the identification of *Enterobacteriaceae*. The results on LIA must be interpreted together with other tests for a correct identification of the strains. Therefore, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; 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.
- 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.
- Apply Good Manufacturing Practice in the preparation process of plated, tubed, bottled media.
- 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 plates 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 applied (temperature and packaging).

16 - REFERENCES

1. Edwards, P.R., and M.A. Fife. 1961. Lysine-Iron Agar in the detection of Arizona cultures. Appl. Microbiol. 9:478-480.
2. Johnson, J.G., L.J. Kunz, W. Barron, and W.H. Ewing. 1966. Biochemical differentiation of the Enterobacteriaceae with the aid of Lysine-Iron-Agar. Appl. Microbiol. 14:212-217.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
4. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev 12/2019

TABLE OF APPLICABLE SYMBOLS

REF or 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	2020/06
Revision 2	Update of "precautions and warnings" and "storage conditions and shelf life"	2022/01
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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