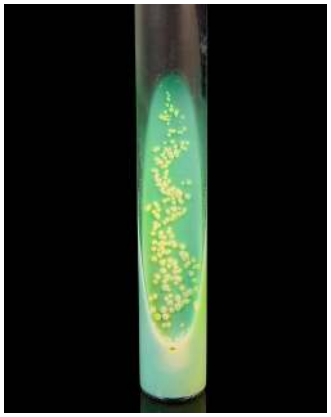
INSTRUCTIONS FOR USE

LÖWENSTEIN-JENSEN MEDIUM BASE

Dehydrated culture medium



M.kansasii
on Lowenstein-Jensen Medium

1 - INTENDED USE

In vitro diagnostic. For the cultivation and isolation of *Mycobacterium* species, especially *M.tuberculosis*.

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

Magnesium sulphate	0.24 g
Magnesium citrate	0.60 g
Monopotassium phosphate	2.50 g
L-asparagine	3.60 g
Potato flour	30.00 g
Malachite green	0.40 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

The medium originally described by Löwenstein in 1931[1] contained congo red and malachite green to limit the growth of unwanted bacteria. In 1932 Jensen[2], modified the medium by suppressing the congo red, modifying the concentration of magnesium citrate and potassium phosphate and increasing malachite green concentration. Today it is generally accepted that the use of an egg-based medium in combination with a liquid medium is essential for good laboratory practice in the isolation of mycobacteria;[3] among the egg-based media Lowenstein-Jensen Medium is the most commonly used in clinical laboratories.
In the Lowenstein-Jensen Medium, during the cooking process, the egg albumin coagulates thus providing a solid surface for bacterial growth. The concentration of malachite green is selected to maximize the growth of mycobacteria while inhibiting other microorganisms. L-asparagine and potato flour are sources of nitrogen and vitamins. Monopotassium phosphate and magnesium sulphate enhance organism growth and act as buffers. Egg suspension provides fatty acids and proteins required for the metabolism of mycobacteria. Glycerol is a carbon source and is favourable to the growth of the human type tubercle bacillus while being unfavourable to the bovine type.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 37.4 g in 600 ml of purified water, add 12 mL of glycerol and heat to boiling. Autoclave at 121°C for 15 minutes. Cool to 50°C and add 1000 mL homogenised whole eggs aseptically collected. Distribute into sterile tubes and heat to 85°C in a slanting position for 45 minutes or more until the medium solidifies due to coagulation of the egg (long slant, short butt).

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	from pale to dark green-blue, fine, homogeneous, free-flowing powder
Solution appearance	green, opaque
Final pH at 20-25°C	not applicable

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Lowenstein-Jensen Medium Base	Dehydrated medium	4016352	500 g (8 L) 21,4
		4016354	5 Kg (80 L) 214

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops, needles and swabs, incubator and laboratory equipment as required, microbiological tubes, Erlenmeyer flasks, chicken eggs, glycerol, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Specimens submitted for mycobacterial culture fall into two categories:
1- specimens normally contaminated with resident flora: the majority originates from respiratory tract, including sputum, tracheal and bronchial aspirates, and bronchoalveolar lavage specimens; other commonly submitted specimens types include urine, gastric aspirates, tissues, biopsy specimens.
2- specimens from normally sterile sites such as pleural and pericardial aspirates.
Contaminated specimens require a decontamination step before culture to reduce the likelihood of overgrowth by organisms other than mycobacteria. Specimens from normally sterile sites should be concentrated by centrifugation. Consult appropriate references for the applicable techniques[3,4] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied.[3,4]

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9 - TEST PROCEDURE

Remove any condensation water present at the bottom of the slope and inoculate the surface of the slope with 0.2 mL (3-5 drops) of decontaminated and/or concentrated specimen.
Briefly angle slopes to allow the specimen to inoculate the entire surface. Ensure that the caps are tightly closed.
Incubate at 35 to 37°C for 6-8 weeks, extending to 12 weeks if necessary. 5-10% CO_2 in air stimulates the growth of mycobacteria in primary isolation cultures. It is necessary to incubate under CO_2, with loosening the caps to promote the circulation of carbon dioxide, for only the first 7 to 10 days after inoculation, subsequently L-J cultures can be removed to ambient air incubators if space is limited and incubated with the caps tightly screwed to prevent dehydration of the medium.[3]
Specimens with positive smear that are culture negative should be held for an additional 4 weeks. The same should be done for culture negative specimens that were positive for mycobacteria by nucleic acid-based amplification assays.[3]
The cultures should be examined within 2 to 5 days after inoculation to permit early detection of rapidly growing mycobacteria. Young cultures (up to 4 weeks of age) should be examined twice a week, whereas older cultures could be examined at weekly intervals.[3]
For samples obtained from surface sites, such as skin, or when the clinician suspects the presence of particular mycobacterial species (*M.marinum, M.ulcerans, M.chelonae,* or *M.haemophilum*), it is recommended to inoculate two sets of media, one of which incubated at 35-37°C and one at a lower temperature (30-32°C).
Consult appropriate references for the detailed procedures about the treatment, inoculation and incubation of clinical specimens.[3,4]

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies
M.tuberculosis appears as granular, rough, dry colonies; *M.kansasii* appears as smooth to rough photochromogenic colonies; *M.gordonae* appears as smooth yellow-orange colonies; *M.avium* appears as smooth, colourless colonies; *M.smegmatis* appears as wrinkled, creamy white colonies.[5]
Confirm the presence of Acid-Fast Bacilli in positive cultures with the Ziehl-Nielsen or auramine-phenol stain.[4]

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.[6]

Control strains	Incubation T°/ t / ATM	Expected results
M.tuberculosis H37Ra ATCC 25177	35-37°C / <21 days / CO_2	growth
M.kansasii Group I ATCC 12478	35-37°C / <21 days / CO_2	growth
M.intracellulare Group III ATCC 13950	35-37°C / <21 days / CO_2	growth
M.fortuitum Group IV ATCC 6841	35-37°C / <21 days / CO_2	growth

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 Lowenstein Jensen Medium is tested for productivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by inoculating Lowenstein Jensen Medium slopes with pure culture of the following target strains: *M.tuberculosis* H37Ra ATCC 25177, *M.kansasii* Group I ATCC 12478, *M. scofulaceum* Group II ATCC 19981, *M.intracellulare* Group III ATCC 13950 and *M. fortuitum* Group IV ATCC 6841.The mycobacteria cultures are incubated at 35-37°C in a 5-10% CO_2 atmosphere and the extend of growth and the colonies' characteristic are recorded after 5 days. All target strains grow with typical colonies.

13 - LIMITATIONS OF THE METHOD

- To shorten the isolation time as much as possible and to obtain a faster identification, the combination of a solid medium and a liquid medium is strongly recommended. The latter allows to reduce the incubation time and egg-based media allow the growth of some strains of *M. tuberculosis* complex and some non-tuberculous species that are unable to develop in liquid media.[7]
- It should be noted that if there is not enough specimen volume for PCR and culture, then only culture should be done. All samples, even if PCR positive, should be submitted for culture.[4]
- M.*bovis* grows poorly, or not at all on L-J medium but grows much better in media where glycerol is substituted by sodium pyruvate.[8]
- *M.leprae* and *M.genavense* fail to grow on L-J Medium.[3,8]
- A negative culture does not exclude an ongoing mycobacterial infection. There are several factors that can be responsible for negative cultures even in the presence of an infection: unrepresentative sample, mycobacteria destroyed during digestion and decontamination of the sample, presence of contaminants that mask or inhibit the growth of mycobacteria, inadequate incubation conditions.
- False positive cultures may result from mislabelling, specimen switching during handling, specimen carryover, contaminated reagents, or cross-contamination between cultures tubes.[3]
- L-J medium contains malachite green and is photosensitive and should not be exposed to light during storage.[8]
- L-J Medium may display some variation in the light-green colour throughout the tube. This doesn't interfere with the growth of mycobacteria; however, colour changes showing bright yellow or dark blue zones may indicate contamination.[8]
- The presence of yellow granules due to the lipid part of the egg, does not interfere with the performance of the medium.
- It is recommended that suitable identification and susceptibility tests be performed on isolates. For the detailed procedures consult appropriate references.[3,4,9]
- 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.
- This culture medium is classified as dangerous; before use, consult the Safety Data Sheet.
- Apply Good Manufacturing Practice in the preparation process of tubed or 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 tubes 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 period of validity of the finished products, according to the type (tubes/bottles), the added supplements and the storage method applied (temperature and packaging).

16 - REFERENCES

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8. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
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401635 LÖWENSTEIN-JENSEN MEDIUM BASE
SDS rev 4
Regulation (EU) 2020/878

Classification

Eye irritation, category 2	H319	Causes serious eye irritation.
Hazardous to the aquatic environment, chronic toxicity, category 3	H412	Harmful to aquatic life with long lasting effects

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Labelling
Hazard pictograms:

Signal words:	Warning
Hazard statements:	
H319	Causes serious eye irritation.
H412	Harmful to aquatic life with long lasting effects.
Precautionary statements:	
P280	Wear eye protection / face protection.
P337+P313	If eye irritation persists: Get medical advice / attention.
P273	Avoid release to the environment.
Contains:	Malachite green oxalate

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 2	Updated layout and content	2022/04
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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