LOWENSTEIN-JENSEN

REF 55244 – 69675

MEDIUM FOR THE ISOLATION OF MYCOBACTERIA

IVD CE

1- INTENDED USE

Lowenstein-Jensen agar is a selective medium well adapted for the isolation, enumeration and differentiation of mycobacteria.
This medium can be used for:

- Primary culture and isolation from pathological specimens of *M. tuberculosis* and atypical mycobacteria.
- Determination of the susceptibility of mycobacteria to specific antibiotics. For this purpose, the medium is first impregnated with increasing concentrations of antibiotics (before coagulation of the medium by heating at 85°C).
- Subculture and preservation of bacillary strains.
- *In situ* biochemical and enzymatic tests used for differentiation of the type of mycobacteria.

2- PRINCIPLE

The selectivity of this medium is based on the presence of malachite green and mineral salts which inhibit the growth of most contaminating organisms.
The growth of mycobacteria is promoted by the nutrients provided, including egg and trace elements.

3- HOW SUPPLIED

- Ready to use medium:
  - 25 x 7 ml screw-top slanted tubes code 55244

- Dehydrated medium:
  - bottle of 500 g code 69675

4- THEORETICAL COMPOSITION (g/l of distilled water)

Lowenstein-Jensen agar is prepared according to the formula described by Lowenstein (1) and modified by Jensen (2).

- Dehydrated medium:

Monopotassium Phosphate	2.4
Magnesium sulphate	0.24
Magnesium citrate	0.6
Asparagine (anhydrous)	3.6
Potato starch	30
Malachite green	0.4

- Ready to use medium:

Dehydrated medium base	
Egg suspension	1000 ml

Preparation of the medium:
Homogenize the powder contained in the bottle.

- Add **37.2 grams** of dehydrated medium to 600 ml of cold sterile distilled water containing 12 ml of glycerol for bacteriology. Do not add glycerol when preparing glycerol-free Lowenstein-Jensen agar.
- Mix until a homogeneous suspension is obtained. Heat gently, shaking constantly, then heat to boiling for 1 to 2 minutes. If necessary, adjust the pH to 6.6. Sterilize in an autoclave at 121°C for 15 minutes.
- Under sterile conditions, prepare 1000 ml of a very homogeneous suspension of whole fresh eggs. Thoroughly wash and disinfect the eggs before breaking them.
- Avoid including air bubbles while breaking and homogenizing the eggs.
- Thoroughly and aseptically mix 600 ml of sterile basic medium, cooled to 45-50°C, and 1000 ml of whole eggs, avoiding the inclusion of air bubbles.
- Dispense the complete medium under sterile conditions into sterile tubes (preferably screw-top tubes).
- Coagulate in an inclined position, in a water-bath or autoclave at 85°C for 45 minutes. Tubes must be stored at +4°C in darkness, while avoiding drying.

5- STORAGE

- Ready to use medium: +2-8°C, horizontally and in darkness
- Dehydrated medium: tightly closed bottle in a dry place at +15-25°C.

The expiry date and batch number are indicated on each packaging.

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6- PROCEDURE

Material:

- Material provided: Lowenstein-Jensen medium.
- Specific material not provided:
  - Plastic cap
  - Glycerol for bacteriology

Precautions for use / Hygiene and safety instructions:

When handling biological specimens likely to contain mycobacteria, prevention measures (3, 4) must be used at all times in compliance with current safety standards for class III micro-organisms.

- All biological specimens can be inoculated on this medium after preliminary liquefaction and decontamination. Refer to current recommendations for storage of biological specimens (5).
- As the specimen may contain only a small quantity of bacteria, it is concentrated by centrifugation to increase the sensitivity of detection.

Inoculation:

Prepare a suspension by adding 3 ml of sterile distilled water to the homogenized centrifugation pellet rich in bacilli and inoculate 0.2 ml of suspension per tube.

Incubation:

- Incubate for 28 days at 37°C in a horizontal position.
- After disappearance of the inoculum (3 or 4 days), close the tubes tightly with the screw-top or a plastic cap.

Reading:

- Enumeration and morphological examination of the colonies should b performed between the 2nd and 8th week after inoculation.
- Colonies of tubercle bacilli only take on a typical appearance when the medium is well oxygenated and the liquid part of the inoculum has fully evaporated. Tubes should only be closed after complete evaporation.

7- PERFORMANCE/QUALITY CONTROL OF THE TEST

- Appearance of the ready to use medium: **pale green** opaque agar slope.
- Appearance of the dehydrated medium: **green**-**blue** powder.
- The growth performances of Lowenstein-Jensen medium are verified with the following strains:

STRAINS	CULTURE RESULTS AFTER 28 DAYS at 37°C
Mycobacterium tuberculosis H 37RV – CIP 64.31	Good growth
Mycobacterium aurum Rebuffet strain	Good growth

8- QUALITY CONTROL OF THE MANUFACTURER

All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to the final commercialization of the product. Each lot is submitted to quality control assessments and is only released to the market, after conforming to pre-defined acceptance criteria. The records relating to production and control of each single lot are kept within Bio-Rad.

9- LIMITS OF USE

- Apart from mycobacteria, Nocardia also grow on Lowenstein-Jensen medium (6).
- It is preferable to use Coletsos medium for cultures of M. bovis.
- Absence of growth does not necessarily mean absence of mycobacterial infection. Certain factors can prevent the growth of mycobacteria.
- It is recommended to perform a sterility test on a sample of each batch of medium prepared from the dehydrated medium.
- Complementary tests must be performed to identify the species of the strain isolated.

10- REFERENCES

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2. JENSEN K.A., Acta Tub. Scand., 1940, **14**, 125.
3. Mesures techniques de prévention, notamment de confinement, à mettre en œuvre dans les industries et les laboratoires de recherche et d'enseignement où les travailleurs sont susceptibles d'être exposés à des agents biologiques pathogènes - Arrêté du 13 août 1996 - Journal Officiel de la République Française.
4. Kent P.T., and KUBICA G.P. 1995. Public health mycobacteriology a guide for the level III laboratory. USDHMS, Centers for Disease Control, Atlanta.
5. Basic Laboratory Procedures Clinical Bacteriology. World Health Organization. Geneva. 1991. 1st edition.
6. HOSTY, T.J. *et al*., J. Clin. Med., 58 : 107, 1961.

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