

Lowenstein-Jensen/Agar (Base for medium)

355-5244
356-9675

DEFINITION

Selective medium for the isolation, enumeration and differentiation of Mycobacteria and to determine the susceptibility of these bacteria to chemotherapeutic agents and antibiotics.

PRESENTATION

• Ready-to-use tubes

7 ml x 25 inclined/screw-capped

code 355-5244

• Dehydrated

500 g

code 356-9675

STORAGE

- Ready-to-use: + 2°C to 8°C.
- Dehydrated: + 15°C to 25°C, in carefully-sealed bottles in a cool, dry place.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Monopotassium phosphate	2.4 g
Magnesium sulfate	0.24 g
Magnesium citrate	0.6 g
Anhydrous asparagine	3.6 g
Potato starch	30 g
Malachite green	0.4 g
Distilled water	1,000 ml

Final pH (25°C) = 6.6 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- Distilled water

EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)

- Hotplate
- Mixer-homogenizer
- Test tubes (16 x 160 mm) with autoclave proof stoppers
- Sterile pipettes (10 ml, etc)
- Thermostatically-controlled incubator or incubation room, precise to ± 1°C
- Autoclave
- All usual laboratory equipment.

PREPARATION OF DEHYDRATED MEDIUM

Always shake well before use.

• Base medium

Dissolve 37.2 g of powder in 600 ml of cold distilled water containing 12 ml of glycerol for bacteriology.

Do not add glycerol if preparing the non-glycerinated Lowenstein-Jensen medium. Mix until a homogenous suspension is obtained. Heat gently, swirling frequently, then bring to boiling point for 1 to 2 minutes. Sterilize in autoclave at 121°C ± 1°C for 15 minutes.

Reconstitution ratio: 62 g/l.

500 g of powder makes 8.06 liters of base medium.

• Complete medium

Under sterile conditions, prepare 1,000 ml of a thoroughly homogenous suspension of whole fresh eggs. Wash and disinfect the eggs before breaking them.

Avoid the creation of air bubbles whilst breaking and homogenizing. Under aseptic conditions, thoroughly mix 600 ml of base medium cooled to 44°C - 47°C and the 1,000 ml of whole eggs, still preventing the inclusion of air bubbles.

Under sterile conditions, dispense the complete medium in sterile tubes (preferably screw-topped).

Coagulate in an inclined position in a water-bath or autoclave at 85°C for 45 minutes. The tubes must be stored at +4°C in the dark, avoiding any desiccation.

It is recommended that a sample of each prepared batch be subjected to a sterility test.

500 g of dehydrated medium makes 21.5 liters of complete medium.

PROTOCOL

• Inoculation and incubation

Primary culture and isolation of *M. tuberculosis* and of atypical mycobacteria.

Determination of the susceptibility of mycobacteria to specific antibiotics: for this use, antibiotics are previously mixed into the medium at increasing levels.

Sub-culture and storage of bacillary strains.

Determination, *in situ*, of biochemical and enzymatic reactions to differentiate type.

READING AND INTERPRETATION

Enumeration, morphological and biochemical study of colonies are performed between the 2nd and 8th week following inoculation.

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PRECAUTIONS

Comply with Good Laboratory Practice.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Results after 28 days culture at 37°C
<i>Mycobacterium tuberculosis</i> H37RV ATCC 25618	Good growth
<i>Mycobacterium aurum</i> Rebuffet strain	Good growth

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product.

Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

Lowenstein - Jensen / Mycobacteria / Isolation / Enumeration / Differentiation / Chemotherapeutic agents / Antibiotics / Medium

BIBLIOGRAPHY

- **LOEWENSTEIN:** Deut. Med. Wsch., (1930), 1010, Bakt., (1931), 120.
- **JENSEN K.A.(1940):** Acta Tub. Scand 14: 125.