OXALIS Diagnostics Pvt. Ltd.

Technical Data

L.J. Medium Slant (7mL)

Recommended for isolation, cultivation and differentiation of mycobacteria especially M. tuberculosis

Product Presentation:

Cat No.	Product description	Pack Size
23010010020	L. J. Medium Slant.	20 Slants

Principle

Lowenstein-Jensen medium base, is used for isolation of acid-fast microorganisms like Mycobacterium species. The base medium is composed of L-asparagine, monopotassium phosphate, magnesium sulphate, magnesium citrate, potato starch soluble and malachite green. L-asparagine is source of nitrogen. Mono-potassium phosphate and magnesium sulfate enhance organism growth and act as buffers. Potato starch is source of carbon and absorb toxic metabolites, malachite green is used to prevent the growth of most contaminants and encourages the growth of mycobacteria. For better result the media can be fortified with egg suspension, glycerol, sodium chloride, penicillin and nalidixic acid alone or in combination, Egg suspension, which provides fatty acids and protein required for the metabolism of mycobacteria. Glycerol serves as carbon source. Sodium chloride helps to differentiate rapid-growing mycobacteria from slow growers. Penicillin and nalidixic acid are added to decrease contamination.
Do not add glycerol to the medium if bovine or other glycerophobic strains are to be cultured.

Specimen Collection and Handling

Ensure that all samples are properly labeled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, followthe standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Reagent

L. J Medium Slant is provided as a ready to use slant. It is a standard non-selective inspissated egg based solid medium for the isolation of *Mycobacterium tuberculosis* from biological specimen such as sputum, CSF, urine.

Additional Material Required

Sterile plating loops (10 µL), incubator at 35°C-37°C, biosafety hood with Bunsen burner, activated 2% glutaraldehyde solution, 0.2 mL micropipettes

Specimen Collection and Preparation

Collect specimen prior to use of antimicrobial agent. Wherever possible, indicate clearly that patient is on antitubercular drugs.
CSF: Collect as much as possible in a syringe, clean skin with alcohol before aspirating specimen.
Body fluids: Disinfect the site and collect specimen with aseptic precautions.
Sputum: Collect 5 to 10 mL in a sterile container from an early morning specimen of deep productive cough. For induced specimen use sterile saline. Have patients rinse mouth with water to minimize specimen contamination with food particles, mouthwash or oral drugs.
Urine: As organisms accumulate in the bladder overnight, first morning void provides best yield. Collect midstream clean catch urine, first morning catheterization/ suprapubic taps in sterile containers.

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Specimen Preparation

Proper decontamination and concentration of specimen containing normal microbial flora are crucial to detection of *Mycobacterium tuberculosis*. Specimen obtained from sterile sites such as CSF, peritoneal or pleural fluids do not need decontamination. However, since most specimens for AFB smear and culture are from respiratory tract and mucous traps AFB and protects other organisms from decontamination and concentration, decontamination and liquefaction is a must. Most satisfactory for this purpose is a combination of N-Acety-L-Cysteine (mucolytic agent) and 2% NaOH (decontaminant).

Preparation of Water Tween Solution

- ✓ To 10 mL of sterile distilled water add 40 µL of sterile tween 80 solution.
- ✓ Mix thoroughly by shaking in a swirling direction or by vortexing to homogenise the solution.
- ✓ Use this solution for preparation of dilution.

Directions

- ✓ Bring the Lowenstein Jensen medium slant to room temperature.
- ✓ Label the L.J. medium slant appropriately.
- ✓ Draw 10 µL of the decontaminated and concentrated specimen from the reconstituted pellet with a sterile calibrated loop and plate it on the L.J. medium slant aseptically.
- ✓ For quantitative evaluation prepare bacterial suspension with sterile water tween solution to match McFarland 0.5 standard, dilute this further with sterile water tween solution 1:10000 and Seed 100 µL on the Lowenstein Jensen medium slant aseptically (seed stock consists of approx.-15000 organisms/mL).
- ✓ Close the L.J. slant cap tightly and incubate at 35°C-37°C.
- ✓ Observe for growth weekly till 8 weeks.

Storage and Stability

- ✓ Store Ready to Use L.J. Medium Slant at 2°C-8°C away from direct light.
- ✓ Avoid freezing and overheating.
- ✓ Use before expiry date on the label.

Quality Control

Appearance: Bluish green coloured, opaque, smooth slant
Growth Promotion Test: Growth is observed after an incubation at 35°C -37°C for 2-4 weeks.

Cultural Response :

Organism	Type Culture	Growth	Colony Characteristics	Incubation Temperature	Incubation Period
Mycobacterium tuberculosis (H37Rv strain)	ATCC 25618	Good	Granular, rough, worthy, dry, friable colonies	35°C -37°C	2-4 Weeks
Mycobacterium avium	MTCC 1723	Good	Smooth, non-pigmented colonies	35°C -37°C	2-4 Weeks

Interpretation of Results

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- ✓ Examination of slant for growth after completion of incubation period.
- ✓ Mycobacterium tuberculosis colonies may be detected from third week onwards up to eight weeks. The colonies are characterized by rough granular buff coloured growth, which has an initial size of 1-3 mm and full-grown size of 58 mm.

Remarks

- ✓ Discoloured, dislodged, or contaminated medium should not be used.
- ✓ Improper decontamination and concentration procedure will yield erroneous results.
- ✓ Treat the specimens and used slants by immersing in 2% activated Glutaraldehyde for at least two hours before incineration and disposal.
- ✓ Good laboratory practices and hazard precautions must be observed at all times.
- ✓ In specimens from patients already on antitubercular drugs, the initial growth may be further delayed.
- ✓ Growth on the Lowenstein Jensen slant within the first week post inoculation usually indicates atypical *Mycobacterium* or contamination due to insufficient decontamination of specimen.
- ✓ All culture growth should be characterized based on morphology, AFB stain and biochemical tests.

Warranty

- ✓ This product is designed to perform as described on the label and package insert. The manufacturer disclaims anyimplied warranty of use and sale for any other purpose.

Disposal

Disposal of infectious material and material that comes in to contact with clinical sample must be decontaminated and dispose of by autoclaving or incineration or established laboratory procedures.
User must be ensuring safe disposal of used or unusable preparation of the products.

Reference

1. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
2. Lowenstein E., 1931, Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig., 120:127.
3. Jensen K. A., 1932, Zentralb. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig., 125:222

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