Lowenstein Jensen Medium Base

Intended Use
Lowenstein Jensen Medium Base is a medium supplemented with eggs, used for cultivation and isolation of *Mycobacterium* species, especially *M. tuberculosis*.

Summary
Solid media used for isolation and cultivation of Mycobacteria are either egg-based or agar-based. Egg based media contain whole eggs or egg yolk, potato flour, salts and glycerol and are solidified by inspissation. Of the egg-based media, Lowenstein Jensen Medium is most commonly used. L.J. Medium was originally formulated by Lowenstein, containing congo red and malachite green dyes. Jensen modified Lowensteins medium by altering the citrate and phosphate contents, eliminating the congo red dye and by increasing the malachite green concentration. Gruft further modified L. J. Medium with the addition of two antimicrobics to increase selectivity. This medium supports the growth of a wide variety of Mycobacteria and can also be used for niacin testing.

Principle
Penicillin and nalidixic acid along with malachite green prevents growth of the majority of contaminants surviving decontamination of the specimen while encouraging earliest possible growth of Mycobacteria. RNA acts as stimulant and help to increase the isolation rate of Mycobacteria. Do not add glycerol to the medium if bovine or other glycerophobic strains are to be cultured. Malachite green serves as an inhibitor and also as pH indicator. Formation of blue zone indicates a decrease in pH by Gram-positive contaminants (e.g. Streptococci) and yellow zones of dye destruction by Gram-negative bacilli. Proteolytic contaminants cause localized or complete digestion of medium.

Formula*
Ingredients	**g/L**
Potato Starch, Soluble	30.0
L-Aspargine	3.6
Monopotassium Phosphate	2.4
Magnesium Citrate	0.6
Malachite Green	0.4
Magnesium Sulphate	0.24

*Adjusted to suit performance parameters

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen
Clinical samples

Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the 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.

Directions
1. Suspend 37.24 g of the powder in 600 mL purified / distilled water containing 12 mL glycerol and mix thoroughly. (Do not add glycerol if bovine tubercle bacilli or other glycerophobic organisms are to be cultivated).
2. Boil to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle. Cool to 45°C-50°C.

4. Prepare 1000 mL of sterile whole Egg Yolk Emulsion (204050370100).
5. Add aseptically and mix the egg emulsion base and Gruft Mycobacterial Supplement (204070350005) (if desired) gently to obtain uniform mixture.
6. Dispense in sterile screw cap tubes and arrange tubes in slanted position.
7. Coagulate and inspissate the medium in an inspissator water bath or autoclave at 85°C for 45 minutes.

Quality Control

Dehydrated Appearance: Greenish blue to peacock blue coloured, homogenous, free flowing powder.
Prepared Appearance: A mixture of sterile basal medium (37.24 g in 600 mL distilled water + 12 mL 85% glycerol) and 1000 mL whole egg emulsion, when inspissated coagulates to yield pale bluish green coloured, opaque, smooth slants.
Cultural Response: Cultural characteristics observed in presence of 5-10% carbon dioxide, with added egg emulsion base, after an incubation of 2-4 weeks at 35°C-37°C

Organism (ATCC)	**Growth**	**Colour Characteristics**
Mycobacterium tuberculosis (H37Rv strain)	Good	Granular, rough, warty, dry, friable colonies
Mycobacterium kansasii (MTCC 3058)	Good	Photochromogenic smooth colonies
Mycobacterium avium (MTCC 1723)	Good	Smooth, non pigmented colonies

Interpretation of Results

1. Cultures should be read within 5-7 days after inoculation and once a week thereafter for up to 8 weeks.
2. Record the following observations: -
i) Number of days required for colonies to become macroscopically visible.
ii) Rapid growers have mature colonies within 7 days; slow growers require more than 7 days.
iii) Pigment production White, cream to buff: Non-chromogenic Lemon, yellow, orange, red: Chromogenis
3. Stained smears may show acid fast bacilli, which are reported only as “acid fast bacilli” unless definitive tests are performed.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:
1. Directions
2. Storage
3. Expiry

Precautions / Limitations

1. Biosafety Level 2 practices, containment equipment and facilities are required for non-aerosol producing activities such as preparation of acid-fast smears.
2. All aerosol generating activities must be conducted in a class 1 or 2 biological safety cabinet. Level 3 practices, containment equipment and facilities are required for laboratory activities in the propagation and manipulation of cultures of *M. tuberculosis* and *M.bovis*.
3. Lowenstein Jensen Base requires incubation in a 5-10% CO_2, atmosphere in order to recover Mycobacteria.
4. The medium should be protected from all sources of light, malachite green is highly photosensitive.
5. Do not use media that have turned yellow, as it will interfere with interpretation of the pigmentation of Mycobacteria. Formation of blue zones indicates a decrease in pH by Gram-positive contaminants like Streptococci and yellow zones of dye destruction by Gram-negative bacilli. Proteolytic contaminants cause localized or complete digestion of the medium.
6. Negative culture results do not rule out active infection by Mycobacteria.
7. Some factors responsible for unsuccessful cultures are:
   i) The specimen was not representative of the infectious material i.e. saliva instead of sputum.
   ii) The Mycobacteria were destroyed during digestion and decontamination of the specimen.
   iii) Gross contamination interfered with the growth of the Mycobacteria.
   iv) Proper aerobic conditions and increased CO_2 tension were not provided during incubation.

Warranty

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

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.
4. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201120270100	Dehydrated Culture Media	100 g
201120270500	Dehydrated Culture Media	500 g