AuSaMicS™ Life Science

TECHNICAL DATA SHEET
AS-1443
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Löwenstein-Jensen Medium Base (L.J. Medium)

Gold-standard egg-based medium for isolation and cultivation of Mycobacterium species, including M. tuberculosis.

OVERVIEW

Löwenstein-Jensen (L.J.) Medium is the most widely used egg-based solid medium for the isolation and cultivation of Mycobacterium species, and remains a cornerstone of tuberculosis diagnostics worldwide. The medium was originally formulated by Ernst Löwenstein in 1931, incorporating congo red and malachite green dyes as selective agents. Jensen (1932) modified the original formulation by altering the citrate and phosphate content, eliminating congo red, and increasing the malachite green concentration — the version in common use today.

L.J. Medium is an egg-based medium: the dehydrated base (supplied as AS-1443) provides the nutrient, buffer, and selective-dye components, but must be combined with glycerol (or sodium pyruvate for glycerophobic strains) and a fresh whole-egg suspension, then solidified by inspissation (heat coagulation), rather than standard autoclave sterilisation. This is a materially different preparation process from conventional agar-based culture media.

PRODUCT IDENTIFICATION

Product Name	Löwenstein-Jensen Medium Base (L.J. Medium)
Catalogue Number	AS-1443
Medium Type	Selective, egg-based solid medium (dehydrated base) — Mycobacterium isolation and cultivation
Reference Formula	Löwenstein E (1931); Jensen K.A. (1932) modification
Dissolution (base)	37.24 g/L base powder in 600 mL purified water containing 12 mL glycerol (per litre of prepared base)
Supplements Required (not in base powder)	Glycerol (12 mL/L base) or sodium pyruvate (for glycerophobic strains); fresh whole egg suspension (1000 mL per 600 mL prepared base)
Final pH	Approximately 6.8 (typical commercial range 6.4–7.0)
Base Sterilisation	Autoclave 121°C for 15 minutes (base solution only, before egg addition)
Final Coagulation	Inspissation at 85°C for 45–50 minutes
Prepared Medium Appearance	Pale bluish-green, opaque, coagulated solid slant

MODE OF ACTION

Potato flour and L-asparagine supply carbohydrate and nitrogen sources respectively, meeting the specific nutritional requirements of Mycobacterium species, which grow slowly and have complex lipid-rich cell walls. Monopotassium phosphate and magnesium sulfate buffer the medium and support general metabolism; magnesium citrate assists in chelating metal ions and improving nutrient availability. Malachite green is the key selective agent: at the concentration used, it inhibits the growth of the majority of contaminating organisms (particularly Gram-positive bacteria) that may survive specimen decontamination, while permitting the growth of acid-fast Mycobacterium species. Malachite green also functions as a built-in pH indicator within the medium — a blue zone indicates a pH decrease caused by gram-positive contaminants (e.g. Streptococci), while yellow zones indicate dye destruction by gram-negative bacilli, both of which are useful contamination indicators independent of the primary target organism.

Glycerol serves as an additional carbon and energy source and favours the growth of the human-type tubercle bacillus (M. tuberculosis), but is inhibitory to M. bovis and other glycerophobic strains — for these organisms, sodium pyruvate is substituted for glycerol. The egg suspension, once heat-coagulated (inspissated), provides essential fatty acids and proteins required for mycobacterial metabolism and creates the solid surface on which colonies develop.

FORMULA — DEHYDRATED BASE, PER LITRE

Component	g/L	Role
Potato Flour (Potato Starch, soluble)	30.000	Carbohydrate/nutrient source
L-Asparagine	3.600	Nitrogen source, essential for mycobacterial metabolism
Monopotassium Phosphate (KH_2PO_4)	2.400	Buffering agent, phosphorus source
Magnesium Citrate	0.600	Magnesium source, metal ion chelation, prevents precipitation
Magnesium Sulfate	0.240	Magnesium source, enzyme cofactor
Malachite Green	0.400	Selective inhibitor of Gram-positive/Gram-negative contaminants; pH indicator

Total dehydrated base: 37.24 g/L.

SUPPLEMENTS ADDED DURING PREPARATION (NOT IN DEHYDRATED BASE)

Supplement	Quantity	Notes
Glycerol	12 mL per litre of prepared base solution (600 mL water + base)	Favours growth of M. tuberculosis (human type); omit for bovine or other glycerophobic strains
Sodium Pyruvate	Substituted for glycerol as required	Used in place of glycerol for cultivation of M. bovis and other glycerophobic Mycobacterium species
Egg Suspension (fresh whole egg)	1000 mL, aseptically prepared	Provides essential fatty acids and proteins; coagulates on heating to form the solid growth surface

PREPARATION PROTOCOL

Step	Instruction
1	Suspend 37.24 g of dehydrated L.J. Medium Base (AS-1443) in 600 mL of purified/distilled water containing 12 mL of glycerol (omit glycerol, substituting sodium pyruvate, for bovine or other glycerophobic organisms).
2	Heat with frequent agitation to completely dissolve the medium.
3	Autoclave the base solution at 121°C for 15 minutes. Cool to 50–60°C.
4	Prepare 1000 mL of a uniform suspension of fresh whole eggs (no older than 3 days) under strict aseptic conditions, avoiding whipping air into the suspension.
5	Aseptically mix the 1000 mL egg suspension with the 600 mL sterile L.J. base solution (cooled to 50–60°C), avoiding the introduction of air bubbles.
6	Distribute the mixture into sterile screw-capped tubes and arrange in a slanted position.
7	Coagulate (inspissate) the medium in an inspissator or water bath at 85°C for 45–50 minutes. Do not sterilise the final egg-containing medium by standard autoclave cycle — inspissation is required for correct coagulation.

INCUBATION & INTERPRETATION

Parameter	Detail
Incubation Temperature	35–37°C, routinely; some protocols recommend triplicate inoculation at 25°C (saprophyte screening) and alternating light/dark at 35°C (pigmentation assessment)
Atmosphere	5–10% CO_2 recommended for optimal recovery

Incubation Duration	Up to 8 weeks; M. tuberculosis is slow-growing (doubling time 15–20 hours) and colonies typically require several weeks to appear
Positive Result	Non-pigmented, rough, dry, buff/cream ("buff, rough and tough") colonies characteristic of M. tuberculosis
Contamination Indicators	Blue zone formation indicates pH decrease from Gram-positive contaminants; yellow zones indicate dye destruction by Gram-negative bacilli; localised or complete medium digestion indicates proteolytic contaminants

APPLICATIONS

Application	Notes
Primary isolation of Mycobacterium tuberculosis	Gold-standard solid culture method for TB diagnosis from clinical specimens (e.g. sputum)
Isolation of non-tuberculous Mycobacteria (NTM)	Recovery and preliminary characterisation of other Mycobacterium species
Niacin testing	L.J. Medium can be used as a substrate for the niacin accumulation test, historically used to help distinguish M. tuberculosis from most other mycobacteria
Drug susceptibility and research applications	Reference medium in mycobacteriology research and susceptibility testing protocols

For laboratory, diagnostic, and research use by appropriately trained and biosafety-accredited personnel only.

STORAGE & STABILITY

Dehydrated Base	Store below 30°C in a tightly closed container, protected from moisture and light. Malachite green is photosensitive.
Prepared Medium (inspissated slants)	Store at 2–8°C, protected from light. Use within the manufacturer's stated shelf life; discard if evidence of contamination, drying, or colour change is observed.
Shelf Life (dehydrated)	Refer to expiry date on label.

LITERATURE REFERENCES

1. Löwenstein E. Ergebnisse und Fortschritte in der Kultur der Tuberkelbazillen aus dem Sputum. Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt 1 Orig. 1931;120:127.

2. Jensen KA. Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt 1 Orig. 1932.

3. Naveen G, Peerapur BV. Comparison of the Lowenstein-Jensen Medium, the Middlebrook 7H10 Medium and MB/BacT for the Isolation of Mycobacterium Tuberculosis (MTB) from Clinical Specimens. J Clin Diagn Res. 2012;6(10):1704–1709.

4. Clinical and Laboratory Standards Institute (CLSI). Laboratory Detection and Identification of Mycobacteria. Approved Guideline.