

For isolation and cultivation of *Mycobacterium* species

### Composition

Ingredients	Gms/Ltr.
Potato starch	30.000
L-Asparagine	3.600
Potassium phosphate mono	2.400
Magnesium sulphate	0.240
Magnesium citrate	0.600
Malachite green	0.400

\* Dehydrated powder, hygroscopic in nature, store in a dry place in tightly- sealed containers 25°C and protected from direct Sunlight.

### Instructions for Use

Dissolve 37.24gms in 600ml distilled water containing 12 ml glycerol. Gently heat to dissolve the medium completely. Sterilize by autoclaving at 15 psi (121°C) for 15 minutes. Prepare 1000 ml of whole egg emulsion collected aseptically. And mix egg and base and **Gruft Mycobacterial supplement (TS 047**, if desired) gently to obtain uniform mixture. Distribute into sterile screw capped tubes. Place tubes in a slanted position in inspissator water bath at 85°C for 45 minutes.

**Appearance:** Pale bluish green coloured, opaque smooth slant  
**pH ( at 25°C):** 7.0 ± 0.2

### Principle

**LOWENSTEIN JENSEN MEDIUM BASE** is used for isolation and cultivation of *Mycobacterium* species. Medium base is a relatively simple formulation that requires supplementation in order to support the growth of mycobacteria. Egg-based media contain whole eggs or egg yolk, potato starch, salts and glycerol and are solidified by inspissations. These substances provide fatty acids and protein required for the metabolism of mycobacteria. The coagulation of the egg albumin during sterilization provides a solid medium for inoculation purposes. Jensen modified Lowenstein 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. 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*. 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. Routinely cultivation is carried out aerobically at 35°C.

#### 1. Lowenstein-Jensen Medium Slants and Bottles:

- a. Inoculation of samples with the culture
- b. By using sterile disposable 0.01ml calibrated loops, inoculate the slants or bottles using *Mycobacterial* cultures prepared as discussed in instructions for use.
- c. Keep the cultures in an upright position for 5 min at room temperature.

## PRODUCT DATA SHEET

- d. Measure (mm) the height of the column of bubbles above the surface of the medium.
- e. Incubate containers with loosened caps at  $35 \pm 2^{\circ}\text{C}$  in an aerobic atmosphere supplemented with carbon dioxide.
- f. Examination of tubes or bottles observed after 7, 14 and 21 days for growth, selectivity and pigmentation.
- g. Inoculate butt surfaces with a sterile disposable 0.01ml inoculating loop using cultures prepared as described above.
- h. Incubate tubes with loosened caps at  $35 \pm 2^{\circ}\text{C}$  in an aerobic atmosphere supplemented with carbon dioxide.
- i. After 14 days incubation, to each culture add 1.0ml of Polysorbate 80 - peroxide mixture.

**For preparing: 10% Polysorbate 80 prepared as follows:**

\*\* Mix 10ml Polysorbate 80 with 90ml purified water. Autoclave at  $121^{\circ}\text{C}$  for 10 min.

\*\* 30% hydrogen peroxide. Store in a refrigerator.

### Interpretation

Cultural characteristics observed after inoculating ( $10^3$  CFU/ml), in the presence of 5-10%  $\text{CO}_2$ , with added egg emulsion base, after incubation at  $35^{\circ}\text{C}$  for 21 days.

Microorganisms	ATCC	Growth with Gruft Supplement	Appearance of colony
<i>Mycobacterium avium</i>	25291	Luxuriant - good	No pigment ( or colourless colonies)
<i>Mycobacterium gordonae</i>	14470	Luxuriant - good	Yellow - orange colonies
<i>Mycobacterium kansasii</i>	12478	Luxuriant – good	Photo chromogenic, smooth to rough
<i>M. tuberculosis H37RV</i>	25618	Luxuriant – good	Granular , warty, dry friable

### References

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