

## Specification

Culture and differentiation of *Mycobacterium* species

## Presentation

	Packaging Details	Shelf Life	Storage
20 Tubes Tube 17 x 145 mm with: 9 ± 0,5 ml	1 box with 20 tubes, 17x145 mm glass tubes, ink labelled, metal-Non injectable cap.	12 months	2-25°C

## Composition

Composition (g/l):	
Potato starch.....	30.0
Asparagine.....	3.60
Magnesium citrate.....	0.60
Magnesium sulfate.....	0.24
Potassium dihydrogen phosphate.....	2.40
Malachite green.....	0.40
Glycerol.....	12.0 ml
Egg emulsion.....	1000.0 ml
Distilled water.....	600.0 ml

## Description /Technique

### Description

Löwenstein originally formulated a medium for cultivation of mycobacteria in which congo red and malachite green were incorporated for the partial inhibition of other bacteria. The present formula, developed by Jensen, differs in the citrate and phosphate content, does not contain congo red and has an increased malachite green concentration.

Lowenstein-Jensen Medium Base is a relatively simple formulation that requires supplementation in order to support the growth of mycobacteria. Glycerol (if required) and egg mixture are added prior to the inspissation process. These substances provide fatty acids and protein required for the metabolisms of mycobacteria. The coagulation of egg albumin during the sterilization provides a solid medium for inoculation purposes.

### Technique

The sample must be treated according its origin and concentrated if it is necessary. All the manipulations with the sample must be performed with the suitable safety standards. Inoculate the culture medium massively by spreading the sample in the surface. Use the glycerol-free culture medium when culturing glycerophobic mycobacteria. Incubate for four weeks at 35°C in horizontal position. After the hiding of the inoculum (2-3 days) the tubes are firmly tightened and aerated weekly. Typical colonial morphology requires a good oxygenation and absence of liquid in the surface. Check the tubes for colony growth after 10-14 days and then in weekly intervals. The final result is obtained after 8 weeks of incubation. Appearance of colonies of *Mycobacterium tuberculosis* on Lowenstein-Jensen Medium with Glycerol or not.

**Type humanus (R variant)** - with glycerol Eugonic growth: Abundant, raised, crumbly, dry, usually yellowish (navel form) colonies - Glycerol-free The same pattern but with a poorly growth

**Type bovinus (S variant)** - with glycerol Sparse growth or no growth at all - Glycerol-free Dysgonic growth: flat, moist, glossy, confluent colonies (often nipple form) without pigment formation.

**Type gallinaceous y Tipo poikilothermorum** - with glycerol and Glycerol-free Rapid growth in the form of a moist, fairly abundant "lawn". Optimal temperature 25°C

Optimal temperature 41-42°C

**Quality control****Physical/Chemical control**

Color : Light green

pH:  $7.2 \pm 0.2$  at  $25^{\circ}\text{C}$ **Microbiological control**

Prepare a suspension from pure culture.

Loop spreading

Aerobiosis. Incubation inclined tubes for a maximum of 21 days

**Microorganism****Growth***Mycobacterium gordonae* ATCC® 14470

Good

*Mycobacterium kansasii* ATCC® 12478

Good

*Mycobacterium tuberculosis* ATCC® 25177

Good

*Mycobacterium fortuitum* ATCC® 6841

Good

*Mycobacterium smegmatis* ATCC® 14468

Good

*Mycobacterium terrae* ATCC® 15755

Good

*Mycobacterium intracellulare* ATCC® 13950

Good

**Sterility Control**Incubation 7 days at  $32.5 \pm 2^{\circ}\text{C}$  and 7 days at  $22.5 \pm 2^{\circ}\text{C}$ : - NO GROWTH**Bibliography**

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