

Specification

Culture and differentiation of *Mycobacterium* spp.

Presentation

	Packaging Details	Shelf Life	Storage
20 Tubes / Slant Tube 16 x 113 mm with: 6,5 ± 0.2 ml	1 box with 20 tubes, 16x113 mm glass tubes, ink labelled and metal cap.	12 months	2-25 °C

Composition

Composition (g / 1600 ml):

Potato starch.....	30.00
Asparagine.....	3.60
Magnesium citrate.....	0.60
Magnesium sulfate.....	0.24
Potassium dihydrogen phosphate.....	2.40
Malachite green.....	0.40
Glycerol.....	12 ml
Egg emulsion.....	1000 ml
Distilled water.....	600 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".

Precautions

For in vitro diagnostic use. Do not reuse. For professional use only.

Do not use if the product is contaminated, broken, or spoiled.

Store in a dark, dry place in their original packaging.

Avoid freezing and overheating.

The expiration date is the date of maximum inoculation.

The greenish color of the medium may change during its useful life. However, should not be used when the medium has a intense blue color, by being exposed to sunlight.

Quality control**Physical/Chemical control**

Color : Light green

pH: 7.2 ± 0.2 at 25°C**Microbiological control**

Prepare a suspension from pure culture.

Isolation by loop spreading

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosis. Incubation inclined tubes for 35-37 °C 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 controlIncubation 7 days at 32.5 ± 2 °C and 7 days at 22.5 ± 2 °C: NO GROWTH.**Bibliography**

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- LOWENSTEIN, E. (1931) Die Züchtung der Tuberkelbazillen aus dem strömenden Blute. Zbl. Bakt. I. Orig. 120:127-129
- BALOWS A., W.J. HAUSLER JR , K.L. HERRMANN, H.D. ISENBERG, H. JEAN · SHADOMY (1991) Manual of Clinical Microbiology 5th ed ASM Press, Washington DC.
- PFYFFER G.E., B.A. BROWN-ELLIOT & R.J. WALLACE Jr. (2003) Mycobacterium: General Characteristics, Isolation, and Staining Procedures in Manual of Clinical Microbiology 8th ed. by Murray, Baron, Jorgensen, Pfaller and Tenover. ASM Press, Washington DC.