Lowenstein Jensen Medium Base w/o Starch

M1542

Lowenstein Jensen Medium w/o Starch is recommended for susceptibility testing of Mycobacteria with addition of antitubercular drugs.

Composition**

Ingredients	Gms / 600ml
L-Asparagine	3.600
Potassium dihydrogen phosphate	2.400
Magnesium sulphate	0.240
Magnesium citrate	0.600
Malachite green	0.400

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 7.24 grams in 600 ml distilled water containing 12 ml glycerol (for bovine bacteria or other glycerophobic organisms, addition of glycerol is not desirable). Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Meanwhile prepare 1000 ml of whole egg emulsion collected aseptically. Add and mix egg emulsion base gently to obtain uniform mixture. Distribute in sterile screw capped tubes. Arrange tubes in a slanted position. Coagulate and inspissate the medium in an inspissator, water bath or autoclave at 85°C for 45 minutes.

Principle And Interpretation

The original LJ medium was formulated by Lowenstein (1) and modified by Jensen (2) and Gruft (3,4) with addition of two antimicrobial agents. Lowenstein Jensen Medium Base w/o starch is recommended for resistance testing by WHO. Lowenstein-Jensen (L-J) Medium without potato starch with drugs incorporated before inspissation is the modification of the International Union Against Tuberculosis (IUAT) (5,6,7).

Malachite green prevents growth of the majority of contaminants that survived the decontamination procedures for the specimen, thus encouraging earliest possible growth of Mycobacteria. Do not add glycerol to the medium if bovine or other glycerophobic strains are to be cultured (8). Malachite green serves as an inhibitor and also as a pH indicator. Formation of blue zone indicates a decrease in pH by gram-positive contaminants (e.g. Streptococci) and yellow zones indicate dye destruction by gram-negative bacilli. Proteolytic contaminants cause localized or complete digestion of medium. Hardy et al (14) recommended each specimen to be inoculated and incubated in triplicate, so as

a. To identify saprophytes at room temperature (25°C).

b.,,To identify presence or absence of pigmentation by photochromogenes and scotochromogenes at 35°C alternately in light and dark as per the type of organism.

Routinely, cultivation is carried out aerobically at 35°C.

Refer appropriate references for standard test procedures of decontamination and isolation (9-13).

Quality Control

Appearance

Greenish blue to peacock blue homogeneous free flowing powder

Colour and Clarity of prepared medium

The mixture of sterile basal medium and whole egg emulsion, when inspissated, coagulates to yield pale bluish green coloured opaque, smooth slants

Cultural Response

M1542: Cultural characterisitics observed in presence of 5-10% Carbon dioxide (CO2), with added egg emulsion base, after an incubation at 35-37°C for 2-4 weeks.

Organism	Colony characteristics
Mycobacterium avium ATCC 25291	smooth, nonpigmented colonies
Mycobacterium gordonae ATCC 14470	smooth, yellow orange colonies
Mycobacterium kansasii ATCC 12478	photochromogenic, smooth to rough
Mycobacterium smegmatis ATCC 14468	wrinkled, creamy white colonies
M. tuberculosis H37RV ATCC 25618	granular, rough, warty, dry friable colonies

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2- 8°C. Use before expiry date on the label.

Reference

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