Sensicult® Secondary (6 Drugs)

Intended Use

Sensicult® Secondary is a ready to use Lowenstein Jensen solid media containing six secondary drug panels (para-Aminosalicyclic acid, Ciprofloxacin, Amikacin, D-cycloserine, Kanamycin and Ethionamide) for *Mycobacterium tuberculosis* sensitivity test.

Summary

Inadequate chemotherapy, irregularity of treatment and use of improper antitubercular regimen lead to high failure rates of antitubercular treatment. As a result, the prevalence of chronic patients discharging drug-resistant organisms increases. Alarming figures of drug resistance in newly detected patients are being reported, mainly from developing countries. This calls for testing of antibiotic sensitivity *In vitro* prior to starting therapy.

Principle

Due to increase in drug resistant strains of *Mycobacterium tuberculosis* and increasing failure rates of antitubercular drug regimens, it is desirable to start antitubercular therapy only after sensitivity assay of the most suitable drug against particular isolate infecting the patient.

Reagent

Microxpress® Sensicult® L.J. Secondary drug panel are reagents for laboratory use only.
Secondary drug containing Lowenstein Jensen media panel for MTB sensitivity tests is a set of ready to use Lowenstein Jensen solid medium slants incorporated with individual antitubercular drugs of recommended specified strength.

Additional Material Required

Sterile plating loops (10 µL), incubator at 37°C±0.5°C, biosafety hood with Bunsen burner, activated 2% glutaraldehyde solution, vortex mixer, 0.1 mL-1.5 mL micropipettes and sterile micropipette tips.

Directions

1. Bring the secondary drug containing Lowenstein Jensen medium panel for MTB sensitivity tests slants to room temperature.
2. Draw 100 µL of the prepared inoculum with a sterile calibrated loop and plate on each slant of secondary drug containing Lowenstein Jensen medium panel for MTB sensitivity tests.
3. A fresh disposable loop should be used for each slant.
4. Close the cap tightly and incubate at 37°C±0.5°C.
5. Observe for growth weekly till 8 weeks.

Inoculum Preparation for Sensitivity Testing

1. Take a loopful asceptically from the *Mycobacterium tuberculosis* colony grown on Lowenstein Jensen medium slant.
2. Transfer it asceptically to the screw capped bottle containing 0.1 mL of sterile distilled water and glass beads, for inoculum preparation.
3. Close cap tightly and subject the contents of the bottle to mechanical shaking (vortex) for 10 minutes.
4. Keep standing for 10 minutes before opening the bottle.
5. Dilute this in saline to match McFarland 0.5 Standard. This contains approximately 1.5 x 10^8 cfu/mL.
6. Further dilute to 1:1000 with saline. This is seed culture (10000-12000 cfu/mL).
7. Mix well and use this as inoculum.
8. Discard the container with glass beads in 2% activated glutaraldehyde solution.

Contents

Secondary drug Lowenstein Jensen medium panel contains Lowenstein Jensen medium with the following antibiotics/ antitubercular drugs.

Sr. No.	Drug	Symbol	pH	Concentration
1.	p-Aminosalicyclic acid	PA	7.0 ± 0.1	0.5 µg/mL

2.	Ciprofloxacin	CP	7.0 ± 0.1	20.0 µg/mL
Sr. No.	**Drug**	**Symbol**	**pH**	**Concentration**
3.	Amikacin	AM	7.0 ± 0.1	20.0 µg/mL
4.	D-cycloserine	DC	7.0 ± 0.1	30.0 µg/mL
5.	Kanamycin	KA	7.0 ± 0.1	20.0 µg/mL
6.	Ethionamide	ET	7.0 ± 0.1	20.0 µg/mL
7.	L.J. Control	LJ	7.0 ± 0.1	-
8.	Sterile distilled water with glass beads for inoculum preparation			

Quality Control

Appearance:
Lowenstein Jensen control slant- Bluish green coloured, opaque, smooth slant.
para-Aminosalicyclic Acid slant- Bluish green coloured, opaque, smooth slant.
Ciprofloxacin slant- Bluish green coloured, opaque, smooth slant.
Amikacin slant- Bluish green coloured, opaque, smooth slant.
D-cycloserine slant- Bluish green coloured, opaque, smooth slant.
Kanamycin slant- Bluish green coloured, opaque, smooth slant.
Ethionamide slant- Bluish green coloured, opaque, smooth slant
Sterile distilled water with glass beads- Clear colourless liquid.
Cultural Response: Cultural characteristics observed after an incubation of 2-4 weeks at 35°C-37°C.

Organism	**Growth on L.J. Control Slant**	**Colony Characteristics on L.J. Control Slant**	**Growth on Slant with Antibiotics**
Mycobacterium tuberculosis H37Rv strain	Good	Granular, rough, warty, friable dry colonies	Inhibited

Interpretation of Results

As and when there is sufficient growth on control (>100 colonies) compare the growth with the antibiotic containing media.

1. If ratio of the growth in antibiotic containing media as compared to control is less than 0.01, the isolate will be termed as Sensitive.
2. If ratio of the growth in antibiotic containing media as compared to control is more than 0.01, the isolate will be termed as Resistant.

Example:

$$\text{Ratio} = \frac{\text{No. of colonies on antibiotic containing media}}{\text{No. of colonies on control media}}$$

Sensitive - If ratio is less than 0.01
Resistant - If ratio is more than0.01
Borderline - If ratio is equal to 0.01

Remarks

1. Discoloured, dislodged, or contaminated medium should not be used.
2. Good laboratory practices and hazard precautions must be observed at all times.
3. Treat the specimen and used slants by immersing in 2% activated Glutaraldehyde for at least two hours before incineration and disposal.

Storage and Stability

1. Avoid jerks and vibration during storage, shipping and incubation.
2. Store the Sensicult® Secondary kits at 2°C-8°C, away from light.
3. Stability of the unopened medium is as per the expiry date mentioned on the label.
4. Upon opening, the medium must be put into use instantly.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Clinical Diagnosis & Management by Laboratory Methods, Todd, Sanford & Davidsohn, 17th Edition 1998, Edited by John Bernard Henry.
2. Tuberculosis; A Clinical Handbook, 1st Edition 1995, Edited by L.I. Lutwick.
3. Practical Medical Microbiology, Mackie & McCartney, 13th Edition 1989, Edited by J.G. Collee, J.P. Duguid.
4. Microbiology, Zinsser, 16th Edition 1976, Edited by W.J. Joklik, H.P. Willet.
5. Health Organisation, Geneva, 1979.
6. Manual of Clinical Microbiology; 5th Edition, ASM Press., Washington D.C.
7. Bombay Hospital Journal; Drug Resistance in Tuberculosis; by Lina Deodhar *et al.,* April 1999.
8. Gradwohl's Clinical Laboratory Methods & Diagnosis; Edited by A. C. Sonnenwirth & L. Jarett. Vol.2, 8th Edition, 1982.
9. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
203190790001	Ready Prepared Kit	One Set