Sensicult® Secondary (10 Drugs)

Intended Use
Sensicult® Secondary is a ready to use Lowenstein Jensen solid media containing ten secondary drug panels (para-Aminosalicyclic acid, Ciprofloxacin, Amikacin, Kanamycin, Ethionamide, Pefloxacin, Lomefloxacin, Rifabutin, Levofloxacin and Ofloxacin) 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, sterile micropipette tips, sterile screw capped bottle containing 0.1 mL of sterile water tween solution and glass beads.

Directions
I: Ratio Method
1. Bring the secondary drug containing Lowenstein Jensen medium panel for MTB sensitivity tests slants to room temperature.
2. Apply 100 µL from seed stock to each slant of secondary drug containing Lowenstein Jensen media panel for MTB sensitivity tests including the L.J. control also.
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.

II: Proportional Method
1. Bring the secondary drug containing Lowenstein Jensen media panel for MTB sensitivity tests slants to room temperature.
2. Apply 100 µL from seed stock to each slant of secondary drug containing Lowenstein Jensen media panel for MTB sensitivity tests excluding the L.J. control.
3. Dilute the seed stock 1:100 with water tween solution and apply 100 µL on to the L.J. control slant.
4. A fresh disposable loop should be used for each slant.
5. Close the cap tightly and incubate at 37°C±0.5°C.
6. Observe for the growth after 2 weeks till 8 weeks, every week.

Preparation of Water Tween Solution
1. To 10 mL of sterile distilled water add 40 µL of sterile Tween 80 solution.
2. Mix thoroughly by shaking in a swirling direction or by vortexing to homogenize the solution.
3. Use this solution for preparation of dilution.

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 water Tween solution to match McFarland 0.5 Standard. This contains approximately 1.5 x 10^8 cfu/mL.
6. Further dilute to 1:1000 with water Tween solution. 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	2.0 µg/mL
3.	Amikacin	AM	7.0 ± 0.1	4.0 µg/mL
4.	Kanamycin	KA	7.0 ± 0.1	30.0 µg/mL
5.	Ethionamide	ET	7.0 ± 0.1	40.0 µg/mL
6.	Pefloxacin	PF	7.0 ± 0.1	2.0 µg/mL
7.	Lomefloxacin	LO	7.0 ± 0.1	5.2 µg/mL
8.	Rifabutin	RF	7.0 ± 0.1	0.5 µg/mL
9.	Levofloxacin	LF	7.0 ± 0.1	2.0 µg/mL
10.	Ofloxacin	OF	7.0 ± 0.1	2.0 µg/mL
11.	Control	LJ	7.0 ± 0.1	-
12.	Control	LJ	7.0 ± 0.1	-

Quality Control

Appearance:
Lowenstein Jensen control slant- Bluish green coloured, opaque, smooth slant.
p-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.
Kanamycin slant- Bluish green coloured, opaque, smooth slant.
Ethionamide slant- Bluish green coloured, opaque, smooth slant.
Pefloxacin slant- Bluish green coloured, opaque, smooth slant.
Lomefloxacin slant- Bluish green coloured, opaque, smooth slant
Rifabutin slant- Bluish green coloured, opaque, smooth slant
Levofloxacin slant- Bluish green coloured, opaque, smooth slant
Ofloxacin slant- Bluish green coloured, opaque, smooth slant
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
Mycobacterium avium MTCC 1723	Good	Smooth, non-pigmented colonies	Inhibited
Mycobacterium kansasii MTCC 3058	Good	Smooth-rough, photochromogenic colonies	Inhibited

Interpretation of Results

I: For Ratio Method

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

II: For Proportional Method

As and when there is growth on control check the growth on antibiotic containing media.

1. If growth observed on antibiotic containing media it is termed as RESISTANT.
2. If no growth observed on antibiotic containing media it is termed as SENSITIVE.

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. Tuberculosis case finding and chemotherapy, K. Toman, World 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. Diagnostics Microbiology, Bailey and Scotts.
10. Laboratory methods for clinical and public health Mycobacteriology, U.S. Dept. Of Health, Education and Welfare.
11. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
203190800001	Ready Prepared Kit	One Set

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.