Sensivue® Secondary (10 Drugs)

Intended Use
Sensivue® Secondary is a ready to use Lowenstein Jensen nitrate substrate media incorporated with ten secondary drugs (para-Aminosalicylic Acid, Ciprofloxacin, Amikacin, Kanamycin, Ethionamide, Pefloxacin, Lomefloxacin, Rifabutin, Levofloxacin, Ofloxacin) for Drug Susceptibility Testing of *Mycobacterium tuberculosis* with a Nitrate Reductase Assay using Proportion Method.

Summary
The spread of Multidrug Resistant (MDR) strains of *M. tuberculosis* has become a major public health concern since these bacteria often cause incurable disease. Standard methods for Drug Susceptibility Testing (DST) of *M. tuberculosis*, such as the proportion method, the absolute concentration method and the resistance ratio method are used globally. But these methodologies usually depend on culture on solid media and are therefore time consuming.
Nitrate reduction test is a rapid, reliable and inexpensive method and would identify most resistant and sensitive *M. tuberculosis* strains. It is an interesting alternative to existing drug susceptibility methods such as the proportion and the expensive radiometric methods.
Mycobacteria reduce nitrate to nitrite and this property is used in Sensivue® for drug susceptibility application. The results obtained with Sensivue® are available as early as 5 to 7 days and usually by day 14. Thus, affording reporting of results much earlier than conventional media and almost comparable to more sophisticated radiometric methods. For resource poor settings, Sensivue® offers a convenient rapid alternative.

Principle
Sensivue® system uses the W.H.O. recommended proportion method for drug susceptibility tests. *Mycobacterium tuberculosis* can be identified on the basis of its ability to reduce nitrate present in the L.J. nitrate substrate medium. The presence of nitrite in the media is indicated by the formation of a pale pink or deep red or violet colour on addition of Nitrite detection strip. This principle is utilized for the determination of drug susceptibility. Reduction of nitrate to nitrite or nitrogenous gases in the L.J. nitrate substrate medium incorporated with drug indicates resistance of the isolate to the particular drug at the concentration specified. The results obtained by nitrate reductase assay is much earlier than by visual detection of colonies on conventional media.

Reagent
Microxpress® Sensivue® is a set for laboratory use only.
Sensivue® comprises of 1 set of:
1. Ten L.J. nitrate substrate media incorporated with individual secondary anti-tubercular drugs of recommended specified strengths.
2. Three L.J. nitrate substrate drug free media (D7, D10 and D14).

Additional Material Required
Biosafety hood, activated 2% Glutaraldehyde solution, 0.1-1 mL variable pipette and pipette tips, sterile water tween solution or sterile isotonic saline, incubator, screw cap test tubes (16 X 125 mm), Vortex mixture, sterile water tween solution or sterile isotonic saline, sterile 1 mL glass bottle with glass beads, McFarland Standard No.1, incubator, Nitrite detection strip, Zinc dust reagent (available as Nitrate Reduction Kit from Microxpress®).

Specimen Collection and Preparation
Collect specimen prior to use of antimicrobial agent. Whenever possible, indicate clearly that the patient is on antitubercular drugs.
Culture: 3-4 weeks old cultures obtained from solid media only should be used for testing.

Directions for Indirect Drug Susceptibility from Smear Positive Specimen
1. Draw a loopful of bacterial colonies from fresh cultures obtained on an L.J. medium slant.
2. Transfer these colonies into a sterile 1 mL glass bottle containing 0.1 mL of sterile water Tween solution or sterile saline with glass beads.

3. Cap the bottle tightly and subject the contents of the bottle to mechanical shaking (vortex) for 10 minutes to homogenize the suspension.
4. Keep standing for 10 minutes before opening the bottle.
5. Dilute the turbidity obtained to match McFarland Standard No. 1.
6. Bring the Sensivue® kit to room temperature and label all the vials with the corresponding patient id.
7. Using a pipette, inoculate 0.2 mL of prepared inoculum onto each of the secondary drug nitrate slants.
8. Dilute 1:10 times the inoculum with sterile saline or sterile water tween solution.
9. Using a pipette inoculate 0.2 mL of 1:10 diluted inoculum onto each of the three-drug free L.J. nitrate slants (D7, D10 and D 14). These tubes serve as growth controls.
10. Cap the vials and incubate at 37°C±2°C.
11. On day 7, use the Nitrate Reduction Kit to test the growth obtained.
12. Dip the fresh unused nitrite detection strip directly in the water of condensation of vial D7 only of the Sensivue® kit.
13. Observe for colour change in the water of condensation from colourless to pale pink or deep red or violet.
14. If colour change is observed, test the corresponding L.J. nitrate substrate slants incorporated with secondary drugs on the same day. This is done by dipping Nitrite detection strip directly in the water of condensation of each vial. A fresh unused nitrite detection strip should be used for each inoculation.
15. If no colour change is seen in control vial D7, discard D7 and repeat the procedure for nitrate reductase assay on day 10 using vial D10 and if needed also on day 14 using vial D14.

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.

Contents

Sr. No.	Slant	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.	Rifambutin	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.	L.J. Control Day 7	D7	7.0 ± 0.1	-
12.	L.J. Control Day 10	D10	7.0 ± 0.1	-
13.	L.J. Control Day 14	D14	7.0 ± 0.1	-

Quality Control

Appearance:

Lowenstein Jensen control day 7 slant- Bluish green coloured, opaque, smooth slant.
Lowenstein Jensen control day 10 slant- Bluish green coloured, opaque, smooth slant.
Lowenstein Jensen control day 14 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	Results
Mycobacterium tuberculosis H37Rv strain	1. Nitrate strip when dipped in L.J. control slant should show pink colour after 14 day of incubation at 35°C-37°C 2. No pink colour development in nitrate strip when dipped in L.J. slant with drugs after 14th day of incubation at 35°C-37°C

Interpretation of Results

An isolate is resistant: If the colour change in the respective drug slant is greater than that in the control on the same day.

An isolate is sensitive: If the colour change in the respective drug slant is equal to or less than that in the control on the same day.

Remarks

1. Sensitivity or resistance to a particular drug can be interpreted only when the control slants (D7, D10 or D14) shows colour change.
2. The test can be read after 7 days of inoculation for major of the isolates.
3. The ability to reduce nitrate is typical for *M. tuberculosis*, although some other mycobacterial species, like *M. kansasii* and most rapid growers share these characteristics.
4. Nitrate reductase-negative strains of *M. tuberculosis* are rare (<1%) and would create no false results since the control vials (D7, D10 and D14) would be negative and the test would therefore be invalid.
5. Isolates may reduce nitrate to nitrite and further reduce nitrite to nitric oxide. To confirm the complete reduction of nitrate to nitrite and further to nitric oxide in the negative control vials (D7, D10 and D14), add a pinch of zinc dust in the water of condensation. No change in colour even after addition of zinc dust indicates the growth of the isolate and the test is positive for nitrate reductase assay.
6. Discoloured, dislodged or contaminated culture medium should not be used for testing.
7. The test procedure must be carried out under a biosafety hood.
8. Discard the screw caps tubes, media following good laboratory practices, once the results are read and recorded.
9. Treat the specimens, equipment's and used slants by immersing in 2% activated Glutaraldehyde for at least two hours before incineration and disposal.
10. Good laboratory practices and hazard precautions must be observed as all times.
11. All culture growth should be characterized based on morphology, AFB stain and biochemical tests.

Storage and Stability

1. Store the Sensivue® Secondary kit at 2°C-8°C, away from light.
2. Stability of the Sensivue® Secondary kit is as per the expiry date mentioned on the label.

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. K.A. Kristian Angeby; Rapid and inexpensive drug Susceptibility testing of Mycobacterium tuberculosis with a Nitrate reductase assay; Journal of Clinical Microbiology, February 2002, pp. 553-555.
2. Clinical Diagnosis & Management by Laboratory Methods, Todd, Sanford & Davidsohn, 17th Edition 1998, Edited by John Bernard Henry.
3. Tuberculosis; A Clinical Handbook, 1st Edition 1995, Edited by L.I. Lutwick.
4. Mycobacteriology; Laboratory methods for clinical and public health; U.S. department of health, education and welfare, Public Health Service Publication No. 1547.
5. Procedures for the isolation and identification of Mycobacteria; U.S. department of health, Education, and Welfare, C.D.C., 1975 Edition.
6. Guidelines for speciation within the Mycobacterium tuberculosis complex, 2nd edition, John M. Grange, W.H.O
7. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
203190840001	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.