PNB Sensitivity Test

Intended Use

PNB Sensitivity Test is a ready to use Lowenstein Jensen solid media with para-nitrobenzoic (PNB) acid for differentiating primary isolates into *Mycobacterium tuberculosis* complex and Non-Tuberculous Mycobacteria.

Summary

The genus *Mycobacterium* is responsible for more suffering worldwide than all other bacterial genera combined. Tuberculosis is caused by members of the *Mycobacterium tuberculosis* complex including *M. tuberculosis*, *M. bovis*, *M. microtic* and *M. africanum*. Tuberculosis in humans is generally caused by the two members of the *Mycobacterium tuberculosis* complex i.e. *M. tuberculosis* and *M. bovis*. Mycobacteria that are not included in the *Mycobacterium tuberculosis* complex are referred to as 'Non-Tuberculous Mycobacteria' or NTM. They have also been called 'Mycobacteria Other Than Tubercule bacilli' or MOTT. The non-tuberculous mycobacteria are a heterogeneous group of pigmented and non-pigmented acid-fast bacilli. Most NTM species are either not pathogenic for humans or are rarely opportunistic pathogens that are more frequently associated with pre-existing underlying disease, especially in immuno-compromised persons.

Identification of the primary isolate as belonging to *Mycobacterium tuberculosis* complex or Non-Tuberculous Mycobacteria (NTM) can be ascertained by performing a few simple tests i.e. tolerance to para-nitrobenzoic acid (PNB), niacin production test, nitrate reduction test, catalase activity at 68°C / pH 7.0, besides observing their growth rate at 25°C and 37°C, photochromogenecity, colony characteristics and morphological appearance and pigment production.

Principle

PNB Sensitivity Test is used to identify if an isolate belongs to *Mycobacterium tuberculosis* complex or Non-Tuberculous Mycobacteria. *M. tuberculosis, M. bovis* and their variants are sensitive to para-nitrobenzoic (PNB) acid and fail to grow on an L.J. medium with PNB. Non-Tuberculous Mycobacteria are generally resistant to PNB and show abundant growth on an L.J. medium with PNB. Microxpress® PNB Sensitivity Test has been designed on this basis.

Reagent

Microxpress® PNB Sensitivity Test is a reagent for laboratory use only. PNB Sensitivity Test is provided as ready to use solid L.J. slants incorporated with para-nitrobenzoic (PNB) acid 500 mg/L. PNB Sensitivity Test is a standard non-selective inspissated egg based solid medium. The medium is used to confirm if a primary isolate is a member of the *Mycobacterium tuberculosis* complex or Non-Tuberculous Mycobacteria. Usually, *M. tuberculosis* and *M. bovis* are frequently associated with tuberculosis in humans. *M. tuberculosis* shows abundant growth on an L. J. medium supplemented with glycerol. On the other hand, *M. bovis* grows well on an L. J. medium containing sodium pyruvate. PNB Sensitivity Test medium is to be used with two drug free L.J. controls containing either glycerol or pyruvate. If the primary isolate is suspected to be *M. bovis*, drug free L.J. controls with sodium pyruvate are to be used. However, if the primary isolate is suspected to be *M. tuberculosis*, drug free L. J. controls with glycerol is to be used.

Additional Material Required

Biosafety hood, 3 mm sterile plating loops, activated 2% Glutaraldehyde solution, 0.1-1 mL variable pipette and pipette tips, screw cap test tubes (16 x 125 mm), vortex mixture, sterile distilled water or sterile isotonic saline, sterile 1 mL glass bottle with glass beads, McFarland standard No. 1, incubator, L.J. slants with glycerol (available as Mycocult® from Microxpress®), L. J. slants with sodium pyruvate (available as Mycocult® PY from Microxpress®).

Specimen Collection

3-4 weeks old cultures obtained from only drug free solid media should be used for testing.

Directions if the Primary Isolate is suspected to be *M. tuberculosis* and *M. bovis*

1. Draw a loopful of bacterial colonies from 3-4 weeks old cultures obtained on a drug free solid medium.
2. Transfer these colonies into a sterile 1 mL glass bottle containing 0.1 mL sterile distilled water or sterile saline with glass beads.
3. Cap the bottle tightly and subject the contents of the bottle to mechanical shaking (vortexing) for 10 minutes to homogenize the suspension.
4. Keep the bottle in standing position for 10 minutes before opening.
5. Dilute the turbidity so obtained to match McFarland Standard No. 1. This is the isolate inoculum.
6. Bring the PNB Sensitivity Test kit to room temperature (20°C-25°C) prior to inoculation. Retrieve one slant/ vial from the PNB Sensitivity Test kit per isolate to be tested.
7. Similarly, also retrieve 2 drug free L.J. control slants containing glycerol (*M. tuberculosis*) and sodium pyruvate (*M. bovis*) per isolate to be tested.
8. Appropriately label the slant of PNB Sensitivity Test and the two drug free L.J. control slants containing glycerol per isolate to be tested with the patient number / I.D.
9. Inoculate 0.1 mL of the isolate inoculum with the pipette onto the following:
   a) One PNB Sensitivity Test vial.
   b) Two drug free L.J. control slants containing glycerol (*M. tuberculosis*) and sodium pyruvate (*M. bovis*)
10. Close all the caps tightly and incubate the three slants as follows:

Slants	Temperature	Incubation Specifications	Examine at
PNB slant	37°C	Internally illuminated incubator	3, 7, 14 and 21 days.
L.J. control slant 1	37°C	Internally illuminated incubator	3, 7, 14 and 21 days.
L.J. control slant 2	25°C	Dark incubator	3, 7, 14 and 21 days.

11. When growth is evident on the drug free L.J. control slant incubated at 37°C, examine all the three slants for pigmentation.

Interpretation of Results

Members of the *Mycobacterium tuberculosis* complex can be identified on the following basis:

1. Those that do not grow within three days at 37°C.
2. Those that do not grow at 25°C.
3. Those that do not grow on the PNB slant.
4. Those that do not produce yellow or orange pigmentations in the dark and even after exposure to light.

Remarks

1. Discoloured, dislodged or contaminated culture media should not be used for testing.
2. Identification of *Mycobacterium tuberculosis* complex should be done by a combination of one or more biochemical tests.
3. Problems with incubation at 25°C may be encountered in tropical regions where the ambient temperature is above 25°C. A refrigerated incubator should be used where available. As an alternative, a water bath within a refrigerator or cold room could also be used.
4. If an internally illuminated incubator is not available, remove the slants from the dark incubator as soon as growth is evident, loosen the caps to release oxygen and expose them to daylight (but not to direct sunlight).
5. Alternately, place the slants at a distance of one meter from a laboratory bench lamp for one hour.
6. Reincubate and examine for pigmentation on the following day.
7. Occasionally, some human strains may give faint growth on the PNB medium.
8. The test procedure must be carried out under a biosafety hood.
9. Once the results are read and recorded, discard the screw cap tubes following good laboratory practices.
10. Treat the specimens, equipment and used slants by immersing in 2% activated Glutaraldehyde solution for at least two hours before incineration and disposal.
11. Good laboratory practices and hazardous precautions must be observed at all times.
12. All culture growth should be characterized based on morphology, AFB stain and biochemical tests.

Storage and Stability

1. Store the PNB Sensitivity Test kit at 2°C-8°C, away from light.
2. Stability of the PNB Sensitivity Test 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. Clinical Diagnosis and 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. Mycobacteriology; Laboratory Methods for Clinical and Public Health; U.S. Department of Health, Education and Welfare, Public Health Service Publication No. 1547.
4. Manual on Isolation, Identification and Sensitivity Testing for *Mycobacterium Tuberculosis*; Edition-2, 1998, Govt. of India, National Tuberculosis Institute, Bangalore, India.
5. Guidelines for speciation within the *Mycobacterium tuberculosis* complex, 2 Edition, John M. Grange, W.H.O.
6. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
203160480006	Ready Prepared Kit	6 Tests

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.