SALUBRIS, Inc.

GATEWAY TO HEALTH, WORLDWIDE

TK PNB KIT®

RAPID CULTURE KIT FOR DIFFERENTIATING MYCOBACTERIUM TUBERCULOSIS COMPLEX AND MYCOBACTERIA OTHER THAN TUBERCULOSIS (MOTT)

Catalogue #: TK 003 **Instructions for Use** **For In Vitro Diagnostic Use**

Product name:

TK PNB KIT®

Product's intended use:

TK PNB KIT® is a rapid culture kit used for differentiating mycobacterial species that belong to *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*) from mycobacteria other than tuberculosis (MOTT).

Summary and explanation of the test:

TK MEDIUM® is a rapid culture medium with multiple dye indicators that permit the early detection of mycobacterial growth. Additionally, ***TK MEDIUM®*** can differentiate mycobacterial growth from contamination. The original red color of the medium turns yellow with the growth of mycobacteria and green in the presence of many other bacterial or fungal species. The color change is easily evaluated by the naked eye or using a low cost but very advanced automated incubator reader, ***MYCOLOR TK®***. When a mycobacterial isolate is obtained from a clinical specimen it is important to know whether it is *M. tuberculosis* or MOTT. In many instances MOTT may not be pathogenic for humans and may not require treatment. If it is determined that the isolated MOTT is the cause of an infection, then the treatment regimen to be applied will usually be different than an infection caused by *M. tuberculosis*. ***TK PNB®*** is a ***TK MEDIUM®*** that contains para-nitro-benzoic acid (PNB). PNB inhibits the growth of mycobacterial species that belong to *M. tuberculosis* complex but not MOTT and thus allows the differentiation of these two groups from each other.

Limitations:

Some species belonging to MOTT may be inhibited by PNB. ***TK PNB®*** can only identify if the mycobacterial isolate belongs to *M. tuberculosis* complex or MOTT, but cannot determine the species name of mycobacteria. Further tests are needed to determine the species of the mycobacterial isolate.

Principles of the procedure:

The color change in ***TK PNB®*** is based on multiple dye indicators and depends on the metabolites and enzymes produced by different species of microorganisms. The color change occurs long before the colonies become visible. ***TK PNB®*** does not contain any radioactive material or fluorescent dye and does not require any scintillation counter, UV light, or other specialized detection systems for evaluation of culture tubes. The growth of a mycobacterial isolate in ***TK PNB®*** is compared with the growth in ***TK MEDIUM®***. If the isolate grows in ***TK MEDIUM®*** and is inhibited by ***TK PNB®***, this will indicate that the isolate may belong to *M. tuberculosis* complex. If the isolate grows on both of the media, this will indicate that the isolate may belong to MOTT.

Ingredients:

TK MEDIUM® contains polypeptides, carbohydrates, salts, dye indicators, vitamins.

TK PNB® contains the same ingredients as ***TK MEDIUM®*** and additionally para-nitro-benzoic acid (750µg/mL).

SUSPENSION TUBE T80® contains 1mm size glass beads in sterile Tween80 solution.

Cautions and warnings:

- FOR *IN VITRO* DIAGNOSTIC USE.
- The tubes should only be opened just before use.
- The caps of the tubes should be closed tightly after inoculation in order to monitor the change in gas content. The change in gas content will lead to a color change in the medium.
- Laboratory procedures involving mycobacteria require special equipment and techniques to minimize biohazards. People who apply these techniques are recommended to have special training in this area. Specimen preparation must be done in a biological safety cabinet. Additional precautions should be taken to reduce the risks of accidental exposure to infectious agents. At a minimum, specimen manipulation should be done in a contained environment having controlled access and a tuberculosis exposure control plan. The locations should have surfaces that can be easily decontaminated using an appropriate topical disinfectant.

General safety precautions:

- Always wear masks and gloves when working with potential biohazard material.
- Work in a laminary flow cabin, biosafety level II, when pipetting the samples.
- Never mouth pipette.
- A refrigerated centrifuge with airtight swinging buckets is recommended for sedimenting bacteria.
- If spills of the contaminated material occur, disinfect with 2.5% hypo-chlorite solution.
- Pathogenic microorganisms including Hepatitis B virus and Human Immunodeficiency Virus (HIV) may be present in specimens. Universal precautions and local laboratory guidelines should be followed in handling all items contaminated with blood or body fluids. If a tube is leaking or is accidentally broken during collection or transport, use the established procedures in your facility for dealing with mycobacterial spills. At a minimum, universal precautions should be employed.
- Tubes should be discarded in an appropriate manner according to biosafety principles.

Storage instructions:

Store at 2 to 8ºC.

Indications of instability or deterioration:

Do not use the medium if a color change to yellow or green is observed prior to inoculation.

Specimen collection and preparation for analysis:

Mycobacterial isolates grown in any culture media (Löwenstein-Jensen, Middlebrook Medium, ***TK MEDIUM®***, ***TK SLC®*** can be tested using ***TK PNB KIT®***. Alternatively, the test can be performed directly using an acid fast bacilli positive (AFB+) clinical sample to save time.

Recommended Procedures:

Inoculations should be done in a biosafety level II cabinet.

Materials provided:

One box contains 3 sets of ***TK MEDIUM®***, ***TK PNB®*** in transparent screw capped glass tubes (16mm in diameter), and ***SUSPENSION TUBE T80®***. The materials are sufficient for tuberculosis or MOTT classification of 3 mycobacterial isolates.

Necessary materials that are not provided:

Biosafety level II cabinet.

Materials and equipment needed for microbiological culture inoculations.

Temperature:

The processing of the samples and inoculation should be done at room temperature.

The incubation of the culture tubes should be done at 37°C. Some ssspecies of mycobacteria may require incubation at different temperatures.

Time restrictions:

Although the effect of the length of time between preparation of the suspension and inoculation of the samples has not been determined, inoculation of the samples immediately after being processed may increase the chance of growing mycobacteria in culture media.

TK PNB KIT®

Application:

1. Prepare a suspension of 1.0 Mc Farland of the mycobacterial isolate using ***SUSPENSION TUBE T80®***.
2. Take one tube of ***TK MEDIUM®*** and one ***TK PNB®***. Write down patient information on the tubes.
3. Remove the tube cap.
4. Inoculate 100µL of the bacterial suspension into each tube. Put the inoculum at the top end of the medium and allow it to slowly migrate downward, slowly wetting the entire surface of the medium.
5. Replace the cap making sure that it is closed tightly (this is very important since the color change in the medium is partly dependent on the consumption of oxygen and production of CO_2. This process can only be monitored in an airtight closed tube).
6. Place the tube into a regular 37°C incubator or ***MYCOLOR TK®***.
7. If using ***MYCOLOR TK®***, enter the necessary information related to the patient and the sample.

Alternatively, the test can be performed directly using an acid-fast bacilli positive (AFB+) clinical sample to save time. Samples prepared using the NALC-NaOH decontamination and concentration procedure are suitable for inoculation to ***TK MEDIUM®*** and ***TK PNB®***. The final pH of the sample prepared using this method should be between 7.4 ± 0.2. It is recommended to use ***MYCOPROSAFE®*** to process the samples. This process guarantees the appropriate pH for inoculation.

Visual evaluation:

TK MEDIUM® and ***TK PNB®*** are easily evaluated visually if an automated incubator-reader, ***MYCOLOR TK®*** is not available and the culture tubes are kept in a regular 37°C incubator. The color of the media should be checked visually on a daily basis.

A change in color from red to yellow will indicate mycobacterial growth. The color change will usually occur before colonies become visible. The test should be evaluated when the color of ***TK MEDIUM®*** turns to yellow. If the color of ***TK PNB®*** remains red, this will indicate that the isolate belongs to the *M. tuberculosis* complex group. If ***TK PNB®*** also turns yellow as ***TK MEDIUM®***, this will indicate that the isolate may be MOTT (see limitations). PNB may slow down the growth of some species of mycobacteria. If ***TK PNB®*** has not turned to yellow when the color change is observed in ***TK MEDIUM®***, it is recommended to wait for an additional 48 hours to evaluate the final color change in ***TK PNB®***. A change in color from red to green will indicate contamination with either fungi or gram-negative bacteria.

Evaluation using ***MYCOLOR TK®***.

The evaluation of color changes in culture tubes is automatically followed by ***MYCOLOR TK®***. The color of all culture tubes and growth curves are easily visualized on the screen.

Important:

Color change in ***TK MEDIUM®*** or ***TK PNB®*** only allows early detection and prediction of the type of organism growing in the culture. When any type of color change occurs, a smear should be prepared from the surface of the media. After acid-fast staining, the smear should be examined under the microscope for the presence of acid-fast bacilli and other contaminating organisms.
The final diagnosis should only be made after examination by experienced personnel.

Quality control:

The following organisms are used for quality control and the following color changes are obtained after incubation at 37°C:

Mycobacterium tuberculosis H37Ra:.. ***TK MEDIUM®***..... Yellow
Mycobacterium tuberculosis H37Ra:.. ***TK PNB®***..... Red
Mycobacterium smegmatis:..... ***TK MEDIUM®***..... Yellow
Mycobacterium smegmatis:..... ***TK PNB®***..... Yellow
Escherichia coli:..... ***TK MEDIUM®***..... Green
Uninoculated medium: .....Red

Limitations of the procedure:

A good homogenization of the bacterial suspension is required before inoculating mycobacteria to ***TK MEDIUM®*** and ***TK PNB®***. It may not be possible to inoculate enough and equal amounts of bacteria into each of these media if the suspension prepared in ***SUSPENSION TUBE T80®*** is not homogeneous. This may create differences in the rate of color change by growth and may lead to incorrect classification of mycobacterial species.

Performance characteristics:

Differentiation of M. tuberculosis complex from MOTT, by using ***TK PNB KIT®*** can be achieved in a much shorter period of time compared to classical culture and biochemical identification methods. Usually the results are obtained between 2 to 14 days depending on the growth rate of the mycobacterial species tested.[1,2]

Bibliography:

1. Kocagöz T., A. Alp, A. Albay. A new rapid non-radioactive medium for culturing mycobacteria, that also enables visually differentiation of mycobacterial growth from contamination. American Society for Microbiology, 100th General Meeting, Los Angeles. May 21-25, 2000.
2. Bicmen C., M. Coskun, G. Senol, N. Florat, T. Kocagoz. Comparison of TK and Löwenstein-Jensen Media for primary culture of Mycobacteria: A preliminary study for a new medium. ECCMID. 13th European Congress of Clinical Microbiology and Infectious Diseases, Glasgow, U. K. May 10-13, 2003.

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