Catalase Detection Kit

Intended Use
Catalase Detection Kit is used for differentiation of isoniazid resistant strains of *Mycobacterium tuberculosis* and *Mycobacterium gastri* from genus *Mycobacterium* based on catalase activity.

Summary
Infection with *Mycobacterium tuberculosis* remains a major public health problem. The epidemic of tuberculosis reflects the failure of public health and social programs towards prompt treatment of infected cases and screening high-risk population. Culture, isolation and sensitivity of *Mycobacterium tuberculosis* from patient groups using standard methods remain the gold standard for *Mycobacterium tuberculosis* detection and effective and swift treatment worldwide.
Many a times, Mycobacterium Other Than Tuberculosis (MOTT) may be the cause of disease in humans and other animals. Various biochemical and biological criteria have been used to identify and differentiate *M. tuberculosis* from MOTT. The identification of Mycobacteria to the species level is important because of the clinical significance; some species are pathogenic while others are not. Knowledge of species is also critical in order to provide adequate patient management because specific antimycobacterial drugs are required against different pathogenic Mycobacteria species.
Differentiation of *M. tuberculosis* is possible on the basis of rate and temperature of growth, colonial characteristics and morphology, niacin and catalase reactions, nitrate activity, pigment production, thiophen-2-carboxylic acid hydrazide (TCH) and drug susceptibility patterns. Most species of Mycobacteria except isoniazid-resistant strains of *M. tuberculosis* and *M. gastri* produce catalase enzyme. Therefore, the catalase test is used for the differentiation of isoniazid resistant strains of *M. tuberculosis* and *M. gastri* from genus *Mycobacterium*.

Principle
Most species of Mycobacteria, with the exception of isoniazid-resistant strains of *M. tuberculosis* and *M. gastri*, produce the intracellular enzyme catalase, Catalase splits hydrogen peroxide into water and oxygen. Catalase can be detected and measured in two ways:
1. Room Temperature Method.
2. Heat Stable (pH 7 / 68°C) Method.

Room Temperature Method:
Untreated catalase enzyme produce by Mycobacteria reduces hydrogen peroxide to water and oxygen. This is observed as bubbling of oxygen, which occurs following the addition of 50-100 µL of the tween-peroxide reagent to the 3-4 weeks old culture growth obtained on the solid plate or slant.

Heat Stable (pH 7 / 68°C) Method:
Certain Mycobacteria loose catalase activity when suspended in pH 7.0 buffer and heated to 68°C. These include *M. tuberculosis* and most members of *M. tuberculosis* complex, *M. bovis*, *M. gastri* and some strains of *M. merinum* and *M. avium* complex. On the other hand, certain isoniazid-resistant strains of *M. tuberculosis* produce a heat-stable catalase activity, and a positive test in this case can be particularly valuable.

Reagent
Microxpress® Catalase Detection Kit is a reagent for laboratory use only.
Catalase detection Kit is a biochemical test for the detection of catalase activity. The kit comprises of:
1. Catalase Reagent (R1)- M/15 phosphate buffer pH 7.0
2. Catalase Reagent (R2)- 10% tween 80 solution
3. Catalase Reagent (R3)- Ready to use 30% hydrogen peroxide (H_2O_2)

Catalase Detection Kit is not ready to use and required the preparation of:
1. Tween-peroxide reagent which has to be prepared by mixing equal volumes of 10 % tween 80 substrate reagent and 30 % hydrogen peroxide.
2. Tween-peroxide reagent is required to be prepared fresh each time, before use.

Additional Material Required
Biosafety hood, sterile plating loops / spade, activated 2% Glutaraldehyde solution, 0.1-1 mL variable pipette and pipette tips, screw cap test tubes (16 X 125 mm), test tube stand, water bath or constant temperature block heater.

Specimen Collection

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

Directions

Preparation of 1 mL Tween-peroxide reagent:

1. Just prior to testing, mix 0.5 mL of R2 reagent to 0.5 mL of R3 reagent in a sterile clean test tube.
2. Gentle swirl to mix.

Note: Tween-peroxide reagent is to be prepared fresh prior to performing the test. After performing the test, the remaining reagent should be discarded.

Room Temperature Method:

1. Add 50-100 µL of the Tween-Peroxide reagent to the 34-week old culture growth obtained on the solid plate or slant.
2. Observe for the formation of bubbles. Formation of bubbles may take 5 minutes in some cases.

Heat Stable (pH 7 / 68°C) Method:

1. Label the required number of screw cap tubes to correspond with the cultures to be tested.
2. Open the screw cap tubes and with a sterile pipette add 0.5 mL of R1 reagent, pH 7.0 to each tube.
3. With a sterile loop / spade, emulsify several colonies from the culture into the buffer (R1 reagent).
4. A fresh disposable loop should be used for each inoculation.
5. Cap the tubes and place the tubes containing emulsified colonies in a water bath or constant temperature block heater at 68°C for 20 minutes.
   (Adherence to time and temperature are critical for obtaining accurate results).
6. After 20 minutes, remove the tubes and allow to cool to room temperature on a test tube stand.
7. Add to each tube 0.5 mL of Tween-Peroxide reagent using a pipette.
8. Observe for the formation of bubbles appearing on the surface of the liquid. Hold tubes for 20 minutes before discarding as negative.

Note: Room Temperature and Heat Stable (pH 7 / 68°C) procedures can be performed on the same slant or plate of the culture. Scrape and remove the colonies for Heat Stable Method prior to performing the Room Temperature test.

Quality Control

Appearance:

R1 Phosphate Buffer- Clear colourless liquid.
R2 10% Tween 80- Very pale yellow slightly viscous liquid.
R3 Hydrogen Peroxide (30%)- Clear colourless liquid.

Organisms (ATCC)	**Results**
At Room Temperature:	
Mycobacterium tuberculosis H37Rv Strain	Bubbles form in 5 minutes
Staphylococcus aureus subsp. *aureus* (25923)	No bubbles formed
At 68°C:	
Mycobacterium tuberculosis H37Rv Strain	Bubbles form in 5 minutes
Staphylococcus aureus subsp. *aureus* (25923)	No bubbles formed

Interpretation of Results

Room Temperature Method

1. Immediate profuse bubbles formed - Positive (rapid).
2. Few slow formation of bubbles - Positive (slow).
3. No bubbling obtained until 5 minutes - Negative.

Heat Stable (pH 7 / 68°C) Method

1. Formation of bubbles - Positive.
2. No bubbling obtained until 20 minutes - Negative.

Performance Characteristics

The performance standards of the above product conform to the NCCLS standards of quality assurance for commercially prepared microbiology products.

Remarks

1. Care should be taken to avoid shaking the tube during reading, since Tween 80 alone may form bubbles when shaken and give a false positive reaction.
2. The test procedure must be carried out under a biosafety hood.
3. Discard the screw caps tubes following good laboratory practices, once the results are read and recorded.
4. Treat the specimens, equipment's and used slants by immersing in 2% activated Glutaraldehyde for at least two hours before incineration and disposal.
5. Good laboratory practices and hazard precautions must be observed as all times.
6. All culture growth should be characterized based on morphology, AFB stain and biochemical tests.

Storage and Stability

1. Store the Catalase Detection Kit at 2°C-8°C, away from light.
2. Stability of the Catalase Detection 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. Diagnostic Key to Mycobacteria Encountered in Clinical Laboratories; Lawrence G. Wayne and Joseph R. Doubek; Applied Microbiology, Vol.:16th No.:6, June 1968, p: 925-931.
2. Clinical Diagnosis and Management by Laboratory Methods, Todd, Sanford & Davidsohn, 17th Edition, 1998, edited stby 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. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
203030350020	Ready Prepared Kit	20 Tests