CATALASE TEST

Principle and Purpose

As a result of metabolic respiratory activities that reduce oxygen (O_2), some bacteria will generate powerful oxidizing agents, e.g., hydrogen peroxide (H_2O_2) and superoxide ion (O_2^-) that are capable of rapid damage to or destruction of cellular constituents. In essence, the bacterium would kill itself if one or more protective mechanisms were not available to eliminate these toxic O_2 compounds. These mechanisms include the enzymes superoxide dismutase, which destroys O_2^-, and either catalase or peroxidase, both which catalyze the elimination of H_2O_2. These enzymes can work cooperatively to remove toxic O_2-related compounds. Superoxide dismutase will convert O_2^- to H_2O_2, which is then broken down into water (H_2O) and molecular oxygen (O_2) by catalase or peroxidase.

The production of catalase and its function can be readily detected in bacteria by simply adding H_2O_2 directly to actively-growing cells. If catalase is present, bubbles of released O_2 will be observed (a positive catalase test). The absence of any bubbles is indicative of a negative catalase result. This test is very helpful in differentiating various bacterial groups. Species of *Streptococcus*, *Enterococcus*, *Staphylococcus*, and *Micrococcus* are generally morphologically similar to one another. However, among these genera, only *Staphylococcus* and *Micrococcus* are catalase positive. In a similar manner, *Clostridium* species differ from the morphologically similar Gram-positive genus *Bacillus* in that the latter is catalase positive. Most members of the Enterobacteriaceae are also catalase positive.

Students will perform the catalase test using two different methods: the tube method and the slide method. Both generate the same results, but in future studies one or other may be preferred given time constraints or resources available.

Learning Objectives

Upon completion of this exercise, a student should be able to:

- Understand the underlying basis of the catalase test;
- Properly conduct the catalase test; and
- Accurately interpret the results of this test.

Materials Required

The following materials are necessary to successfully conduct this exercise:

Organisms - The following organisms should be provided as 24-48 hour-old TSA slant or plate cultures:

- *Enterococcus faecalis* (ATCC 19433) [abbreviated as *E. faecalis*]
- *Micrococcus luteus* (ATCC 4698) [abbreviated as *M. luteus*]
- *Staphylococcus aureus* (ATCC 25923) [abbreviated as *Staph. aureus*]
- *Streptococcus pyogenes* (ATCC 19615) [abbreviated as *Strept. pyogenes*]

Media

- TSA slants

Reagents/Materials

- 3% hydrogen peroxide
- 13 x 100 mm test tubes
- Sterile wooden applicator sticks

Procedure

Students shall review and use the BIOL 3702L Standard Practices regarding the labeling, incubation, and disposal of materials.

1) Obtain four (4) TSA slants and label one for inoculation for each of the following bacterial species: *Strept. pyogenes*, *E. faecalis*, *Staph. aureus*, and *M. luteus*. Include other appropriate information when labeling these tubes.
2) Using aseptic technique, inoculate each of these TSA slants with the appropriate bacterium.
3) Incubate the tubes at 37°C for 18-24 hours.
4) Perform both the tube and slide method as indicated below.

Note: Be sure to perform the slide method prior to the tube method!

Slide Method for Catalase Detection

a. Aseptically transfer some of the growth from the TSA slant of the *Strept. pyogenes* to the surface of a glass slide.
b. Add one drop of 3% H_2O_2 from the vial provided to the cells on the slide being careful not to contaminate the tip of the dropper vial. Observe if any gas bubbles appear, which should occur immediately.

Interpretation of Results: The appearance of gas bubbles indicates a positive catalase reaction, whereas their absence indicates a negative test result (Fig. 1).

Record any observations on the data report sheet attached to this document.

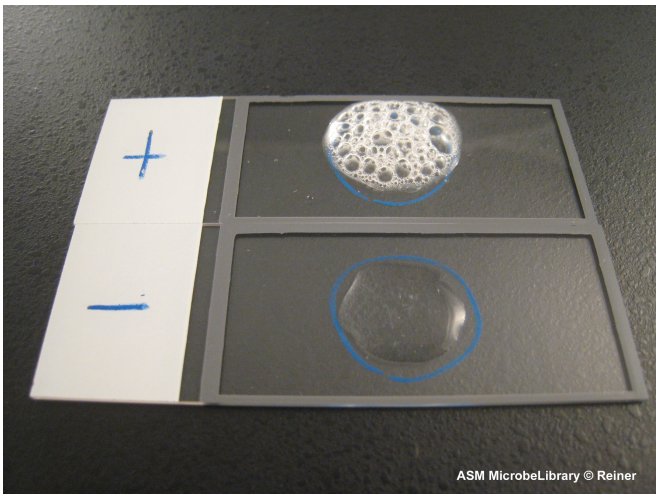

Figure 1. Catalase Slide Test. After adding hydrogen peroxide, bubbling indicates a positive reaction by *Staph. aureus*. Conversely, a negative reaction produced by *Strept. pyogenes* is indicated by the absence of bubbling.

c. Repeat steps 4a and 4b for each of the remaining bacterial species.

Note: The same slide can be used if cross-contamination can be avoided.

Tube Method for Catalase Detection

Two different tube-based approaches are described below. One is analogous to the slide-based methods described above, whereas the other uses an established agar slant culture. The latter method, if used, renders the culture essentially non-viable and should not be used if there is further need of this culture.

Tube Method 1. Obtain four 13 x 100 mm test tubes and label one each as *Strept. pyogenes*, *E. faecalis*, *Staph. aureus*, and *M. luteus*. Into these tubes place 5-7 drops of 3% hydrogen peroxide. Use a sterile wooden applicator stick and retrieve a small amount of cells from a particular TSA slant culture. Place the applicator stick directly into the appropriately labeled test tube containing the hydrogen peroxide. Observe if any gas bubbles appear.

Interpretation of Results: The immediate production of gas bubbles indicates a positive catalase reaction, whereas the absence of bubbles indicates a negative test result (Fig. 2).

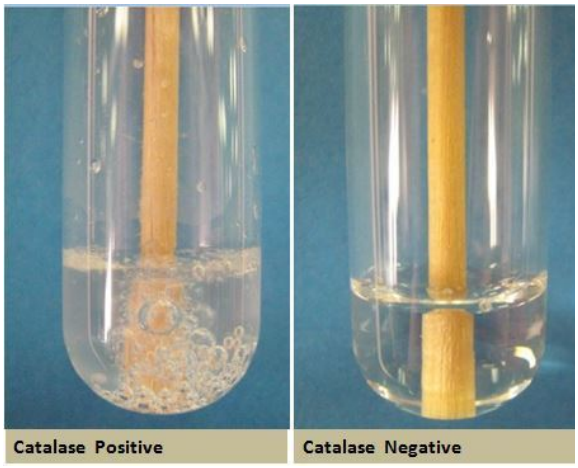

Figure 2. Catalase Tube Test. A wooden applicator containing catalase-positive bacteria inserted in a tube of hydrogen peroxide will generate bubbles (left image). In contrast, a similar test using a catalase-negative microbe does not generate bubbles (left image). Microbeonline.com

Record any observations on the data report sheet attached to this document.

Repeat this procedure for each of the remaining bacterial species.

Tube Method 2. Using one of the TSA slant cultures placed at a slight angle, add 3-5 drops of 3% H_2O_2 from the vial provided over the growth on the slant. Be careful not to contaminate the tip of the dropper vial. Observe if any gas bubbles appear.

Record any observations on the data report sheet attached to this document.

Note: Obviously, once the Tube Method 2 is performed, the TSA slant culture can no longer be used for other purposes.

Interpretation of Results: The immediate production of gas bubbles indicates a positive catalase reaction, whereas the absence of bubbles indicates a negative test result (Fig. 3).

Figure 3. Catalase Culture Tube Test. After adding hydrogen peroxide to a microbial culture, catalase-positive bacteria will generate bubbles. https://laboratoryinfo.com/catalase-test/

Student Name: _____

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Observe and record the results of catalase activity by *Enterococcus faecalis*, *Staphylococcus epidermidis, Streptococcus pyogenes*, and *Micrococcus luteus* when assessed by the tube and slide methods. A bubbling reaction following the addition of hydrogen peroxide is considered a positive result.

Results of Catalase Test*

	Test Method		
Test Organism	**TSA Slant**	**Slide**	**Tube**
Enterococcus faecalis			
Staphylococcus epidermidis			
Streptococcus pyogenes			
Micrococcus luteus			

*The formation of bubbles is a positive test result and should be indicated as "+". The absence of bubbles is a negative test result and should be indicated as "-".

Discussion Question

1) Do bacteria that exhibit an anaerobic life style require catalase? Why or why not?

2) It has been shown that pathogenic microbes that invade and reside within macrophages require the ability to produce catalase and like enzymes. Why?