

Catalase Test

Cells of aerobic and facultatively anaerobic organisms may generate hydrogen peroxide, H_2O_2 , a reactive form of oxygen (Fig. 6-16, p. 50 of the Atlas). H_2O_2 and other reactive forms of oxygen can be very destructive to the cell. One way that cells can remove H_2O_2 is by use of the enzyme catalase (Fig. 6-17, p. 50 of the Atlas). Catalase breaks down H_2O_2 to water and oxygen. The presence of catalase can be tested by adding H_2O_2 to a suspension of cells. The immediate formation of oxygen gas bubbles results if catalase is present.

Note that the presence of catalase may also aid in the survival of pathogens. During an infection, pathogenic bacteria may be phagocytized by macrophage (“big eaters”) cells that are part of the immune system. Phagocytic cells generate reactive forms of oxygen including H_2O_2 as part of a “respiratory burst”. Such reactive forms of oxygen can be generated in sufficient levels to kill the ingested bacteria, however if the bacterium has catalase, one of these reactive forms of oxygen, H_2O_2 , can be degraded back to less destructive O_2 and H_2O . Catalase can be considered a **virulence factor** for such bacterial pathogens because it contributes to the ability of the pathogen to survive in the host organism and cause disease. The presence of catalase does not necessarily mean the bacterium is a pathogen; many aerobic and facultatively anaerobic organisms have catalase yet they are not pathogens.

Procedure:

To demonstrate the Catalase Slide Test, we will use the following test organisms: *Staphylococcus epidermidis* and *Enterococcus faecalis*. Both have been grown on Tryptic Soy Agar (TSA).

1. Place a drop of fresh 3% H_2O_2 on a microscope slide.
2. Flame sterilize a loop and let it cool. Alternatively, a sterile toothpick or wooden stick may be used instead of the loop.
3. Mix in a loopful of the organism to be tested. Mix the suspension thoroughly. (Note, the organism should not be grown on a Blood Agar Plate otherwise a false positive may be obtained.)
4. Look for the almost immediate and vigorous formation of oxygen gas bubbles, that is a positive reaction (Fig. 6-18, p. 51 of the Atlas).
5. Flame sterilize the loop and when finished with all tests, place the slide in the slide disposal dish and cover with Amphyl disinfectant.

Your instructor may also demonstrate the Catalase Tube Test (Fig. 6-19, p. 51 of the Atlas).