DTC Catalase Test

Learning Objectives

The student will

- Use aseptic techniques in the safe inoculation of various forms of media.
- Follow oral and written instructions and manage time in the lab efficiently.
- Apply correct terminology regarding microbiological techniques, instruments, microbial growth, and biochemical testing when making observations.
- Make accurate observations and appropriate interpretations of biochemical test results and use them in the identification of potentially disease causing microbes.

Background/Theory

When an organism uses $O_2$ in respiration, sometimes poisonous hydrogen peroxide, $H_2O_2$, is created. There are three ways this is known to occur.

1. $FADH_2$, carrying electrons from the Krebs cycle, can transfer electrons to $O_2$ skipping the electron transport system (ETS) and producing $H_2O_2$.
2. Flavoproteins, usually the initial electron carriers in the ETS, can transfer electrons directly to $O_2$ bypassing the rest of the ETS. The incompletely reduced oxygen forms $H_2O_2$.
3. In the last step of the ETS, an incomplete reduction can produce the superoxide radical $O_2^-$ which is then converted to $H_2O_2$ by the enzyme superoxide dismutase.

Organisms that produce $H_2O_2$ also produce the enzyme catalase which breaks $H_2O_2$ down into molecular oxygen and water.

$$H_2O_2 \xrightarrow{\text{catalase}} H_2O + \frac{1}{2}O_2 \uparrow$$

To test for the presence of catalase, hydrogen peroxide is dropped onto a mass of cells. If catalase is present, bubbles of $O_2$ form immediately. The reagent can be dropped directly onto cells growing on the surface of a medium. However, you will perform the “slide test,” where cells are first placed on a clean surface and then the reagent is added. *Staphylococcus epidermidis* and *Enterococcus faecalis* will be your controls; one is catalase positive and the other is catalase negative. The two additional organisms of your choice will be your test organisms.

When performing the “slide test” you will use an instrument to transfer cells to a clean surface.

The order of steps is important! Many transfer instruments are composed of metals. Some metals can act as inorganic catalysts and facilitate the same reaction as the catalase enzyme does. If the hydrogen peroxide makes contact with the metal, you could see bubbles because of the interaction of the metal and the $H_2O_2$. This may produce a false positive result. While reading below, think about how the procedure (specific materials used, the order of steps) helps guard against a false positive in this regard.

Experiment/Exercise

**Materials per student pair**

- 1 petri dish
- $H_2O_2$
- Sterile tooth picks

**Cultures**

Fresh overnight plate cultures

*Staphylococcus epidermidis*
Enterococcus faecalis
2 additional organisms from those available this week growing on solid media

Procedure Lab 1
1. On the bottom of the petri dish, divide the surface in quadrants and label each section with an abbreviation for each organism. Turn the plate over so that the lid is on top.
2. Remove the lid. Aseptically, use a sterile toothpick to transfer a visible amount of S. epidermidis growth from the parent culture to the corresponding section of the plate.
3. Discard the toothpick immediately into the disposal container on the bench.
4. In the same way, transfer each of the other three organisms to the plate using a fresh sterile toothpick each time. Take care to keep each sample well away from the other samples on the inside of the plate.
5. Gently add 2 drops of H$_2$O$_2$ solution onto each mass of cells. Take care to keep the reagent from one quadrant rolling into an adjacent area.
6. Place the lid on the plate to contain any aerosols produced by bubbling.
7. Watch for bubbles.
8. Record your data. For observations, briefly describe what you see, “bubbles” or “no bubbles.” Under result, indicate a positive or negative result. Under interpretation, think about what the result tells you about the biochemistry of the organism. The organism “makes catalase” or the organism “does not make catalase.”
9. Keep the plate closed and dispose of it in the plate disposal container.
Lab Report: Catalase Test

Name ______________________________
Lab Section __________

Data and Observations

<table>
<thead>
<tr>
<th>Organism</th>
<th>Observations</th>
<th>Result +/-</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Post Lab Questions

1. What was the positive control? What was the negative control?

2. What two things about the procedure prevent a false positive reaction between a transfer instrument and the reagent?

3. A false positive created by a metal catalyst and the hydrogen peroxide can be described as resulting from poor specificity or poor sensitivity in the test system?
4. Suppose you wanted to test an organism growing on blood agar for the presence of catalase. You perform the slide test as described above and you observe bubbles. You then realize that red blood cells, as aerobic cells, may have catalase activity. At this point, you suspect that you may have an erroneous result.
   a. This could be described as a false ______________.
   b. Describe what you could do to determine if the bubbles were produced by catalase in the bacteria or catalase (or other enzyme) in the red blood cells?
   c. What type of control would that be?

5. What are the negative consequences to the cell producing hydrogen peroxide in the ways described in this exercise? There are at least two; both have something to with ATP production and energy use.
References