

DTO Oxidase Test

Learning Objectives

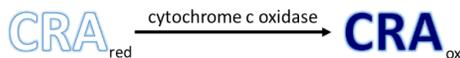
The student will

- Follow oral and written instructions and manage time in the lab efficiently.
- Apply correct terminology regarding microbiological techniques, instruments 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

In **aerobic respiration**, the final electron acceptor (i.e., the one having the most positive redox potential) at the end of the ETS is an oxygen molecule (O₂) that becomes reduced to water (H₂O) by the final ETS carrier. This electron carrier, cytochrome oxidase, differs between bacterial types and can be used to differentiate closely related bacteria for diagnoses. For example, the gram-negative opportunist *Pseudomonas aeruginosa* and the gram-negative cholera-causing *Vibrio cholerae* uses **cytochrome c oxidase**, which can be detected by the oxidase test, whereas other gram-negative Enterobacteriaceae, like *E. coli*, are negative for this test because they produce different cytochrome oxidase types. (OpenStax CNX, 2018)

Microbes that possess cytochrome c, contain cytochrome c oxidase to facilitate electron and proton transfer to oxygen. In this scenario, the cytochrome is the reducing agent because in this step of the ETS, it is the source of the electrons. In the oxidase test, the reagent becomes the source of the electrons. It is a chromogenic reducing agent (CRA). It produces a color change when it gives up its electrons. This reducing agent readily transfers its electrons to cytochrome c oxidase. As the colorless CRA becomes oxidized, it becomes deep purple.



The particular CRA used, tetramethyl-*p*-phenylenediamine, does not transfer electrons to other cytochromes or to other ETS components because its reduction potential is lower than that of the other cytochrome types. The electrons cannot flow “up” the redox tower. The CRA will transfer electrons to cytochrome c oxidase because this enzyme complex has a lower reduction potential and will spontaneously accept them.

In this test, the timing is important. If the moistened reagent is exposed to air for too long, it will spontaneously become oxidized, by substances other than cytochrome C oxidase. When this happens it will change color even when no cytochrome c oxidase is present. Thus, it is very important that you read this test within 20 seconds of applying the reagent. If there is no color change after 20 seconds, the result is negative. Resist the temptation to revise your observation later.

Experiment/Exercise

Materials per student pair

BBL DrySlide
Sterile tooth picks

Cultures

Fresh overnight plate cultures

E. coli

Pseudomonas aeruginosa (Risk Group 2)

2 additional organisms from those available this week growing on solid media

Procedure (BBL DrySlide)

1. You are working with Risk Group 2/BSL-2 organisms. Take care!
2. Place the BBL DrySlide test card on the bench top. Test and record the results for one organism at a time.
3. With a sterile toothpick, aseptically transfer a visible amount of *E. coli* to one window of the card. Note the exact time.
4. Quickly, rub the growth into the filter paper so that it makes good contact with the reagent imbedded in the filter paper. Dispose of the toothpick in the disposal container immediately.
5. Observe for no more than 20 seconds. Look for a color change to dark blue/purple. Record your observation. Does the color remain “unchanged” or does it turn a “dark blue or purple”?
6. Repeat steps 3-5 with *Pseudomonas aeruginosa* next.
7. Repeat steps 3-5 with the other 2 organisms, one at a time.
8. Dispose of the reaction card in the plate disposal tub on the disposal cart.
9. Finish the data table. Under result, indicate “+” or “-”. Under interpretation, indicate what it means. In this case, your interpretation will be that the organism “has cytochrome c oxidase” or it “does not have cytochrome c oxidase.”

Lab Report: Oxidase Test

Name _____

Lab Section _____

Data and Observations

Organism	Observations	Result +/-	Interpretation

Post Lab Questions

1. Where is the reagent in this test?

2. Why were *E. coli* and *Pseudomonas aeruginosa* chosen as your first 2 test organisms?

3. The fact that the CRA will spontaneously give up its electrons after a certain amount of time whether or not cytochrome c oxidase is present can be described as poor _____ in the test system. How does the protocol minimize this possibility for error?

4. Suppose you perform this test on *E. coli* and after you add cells to the reaction card, your partner asks you to help them find cells under the microscope. When you return to the reaction card after 10 minutes, you notice the dark color and record a positive result in your data table.
 - a. You have recorded a false _____
 - b. This an example of poor _____

4. Suppose perform this test on *Pseudomonas aeruginosa* and after you add the cells to the card and wait for 20 seconds, you do not see a color change. After you help your partner find cells under the microscope, you return to see that the reagent has turned dark color.
 - a. How should you record your result as an ethical scientist? _____
 - b. This result is a false _____
 - c. This is an example of poor _____
 - d. What could have caused this result? List at least two possibilities. Be concise.

- e. Why is “contamination” NOT a possible cause?

References

OpenStax CNX. (2018, Mar 19). OpenStax Microbiology. Retrieved from <http://cnx.org/contents/e42bd376-624b-4c0f-972f-e0c57998e765@4.24>