🚊 ELISA

STEM Sims

Lesson 1: Is Fentanyl Present?

ELISA stands for enzyme-linked immunosorbent assay, which is a laboratory tool commonly used in diagnostic medicine used to detect the presence of various antigens or antibodies. ELISA can be used to test a patient's blood or urine for illicit drug use. In this activity, you will test a urine sample for the presence of one of the most dangerous drugs, fentanyl. Make sure to put on your virtual protective gear for this test because even a tiny amount of fentanyl can cause major problems.

Doing the Science

Part I: Standards and Sample Preparation

- 1. Open the ELISA simulation and select practice or test mode based on your instructor's directions.
- 2. Select the marker and move the marker to the microtiter plate on the tabletop to label the plate.
- 3. Select the pipet from the shelf and move the pipet to the "New Tips" area.
- 4. Move the pipet to the "Standard A" container on the shelf. Select the 10 μ L area of the pipet and use the blue arrows to adjust the amount held by the pipet to 20 μ L.
- 5. Depress the plunger to withdraw the liquid; then, move the pipet to the well labeled "A1" on the plate on the tabletop.
- 6. Depress the plunger to add the solution to the plate. Repeat the same process to fill well A2 with Standard A.
- 7. Move the pipet to the waste area to remove the tip.
- 8. Use a similar procedure to fill wells B1 and B2 with standard B, wells C1 and C2 with standard C, wells D1 and D2 with standard D, and wells P1 and P2 with the patient sample. Make sure to place a new tip on the pipet before changing to the next standard or patient sample.
- 9. Select the pipet and move the pipet to the "New Tips" area.

Part II: Reagent Addition

- 10. Move the pipet to the "Enzyme Conjugate" container on the shelf. Select the 10 μ L area of the pipet and use the blue arrows to adjust the amount held by the pipet to 100 μ L.
- 11. Depress the plunger to withdraw the liquid; then, move the pipet to the well labeled "A1" on the plate on the tabletop.
- 12. Depress the plunger to place the solution into well A1. Repeat this process to add 100 μL of the "Enzyme Conjugate" to all of the other wells in the plate.
- 13. Move the pipet to the waste area to remove the tip.
- 14. Select and move the plate to the incubator on the tabletop. Set the timer for 60 minutes and select the "Start" button.
- 15. Select and move the plate to the plate washer on the tabletop.
- 16. Select the pipet and move the pipet to the "New Tips" area.

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- 17. Move the pipet to the "Substrate Reagent" container on the shelf. Select the 10 μ L area of the pipet and use the blue arrows to adjust the amount held by the pipet to 100 μ L.
- 18. Depress the plunger to withdraw the liquid; then, move the pipet to the well labeled "A1" on the plate on the tabletop.
- 19. Depress the plunger to place the solution into well A1.
- 20. Repeat this process to add 100 μ L of the "Substrate Reagent" to all of the other wells in the plate.
- 21. Move the pipet to the waste area to remove the tip.
- 22. Select the plate and move the plate to the incubator. Set the timer for 30 minutes and select the "Start" button.
- 23. Select the pipet and move the pipet to the "New Tips" area.
- 24. Move the pipet to the "Stop Solution" container on the shelf. Select the 10 μ L area of the pipet and use the blue arrows to adjust the amount held by the pipet to 100 μ L.
- 25. Depress the plunger to withdraw the liquid; then, move the pipet to the well labeled "A1" on the plate on the tabletop.
- 26. Depress the plunger to place the solution into well A1.
- 27. Repeat this process to add 100 μ L of the "Stop Solution" to all of the other wells in the plate.
- 28. Move the pipet to the waste area to remove the tip.
- 29. Select and move the plate to the plate reader on the tabletop.

Part III: Results and Evaluation

- 30. Observe the plate and "Mean Absorbance" values for the standards and the patient sample. Record your results in Table 1. If necessary, create a graph to better evaluate your results.
- 31. When ready, select the "Evaluate" button to enter your patient evaluation on screen and record your patient evaluation in Table 1.
- 32. Select the "Check Answer" button if in the practice mode or the "Submit Answer" button if in the test mode.

Table 1. Patient Results and Evaluation.

Patient ID #:

Sample	Concentration (ng/mL)	Mean Absorbance	Well Color
Standard A			
Standard B			
Standard C			
Standard D			
Patient Sample			

Do You Understand?

1. What is the purpose of the antibody in ELISA testing for the drug fentanyl?

2. How is the final solution color related to fentanyl concentration in ELISA testing for the drug fentanyl?