GENERATION HEALTH
How Science Powers Us

Teacher Guide

# ELISA Lung Cancer Lab

Students will be taking on the role of a lab technician at a biomedical research facility to assist a doctor in the state of Maryland in handling patient samples confirmed for lung cancer. Students will receive patient profiles to go along with the patient samples and will be making predictions as to what type of lung cancer each patient has. They will perform an ELISA on the samples and then confirm their predictions.

## WORKSTATION SETUP

Due to the cost of ELISA, we will be having each pair of student work with one set of microplate wells. They will be part of a bigger cohort where each of the samples are there. Six students will have all three serum samples, with pairs working on an individual set of microplate wells. If there is an uneven number of sets of microplate wells due to classroom size, teachers have the option to do three to a set of microplate wells or have cohorts with four pairs instead of three. Give students a copy of the student worksheet.

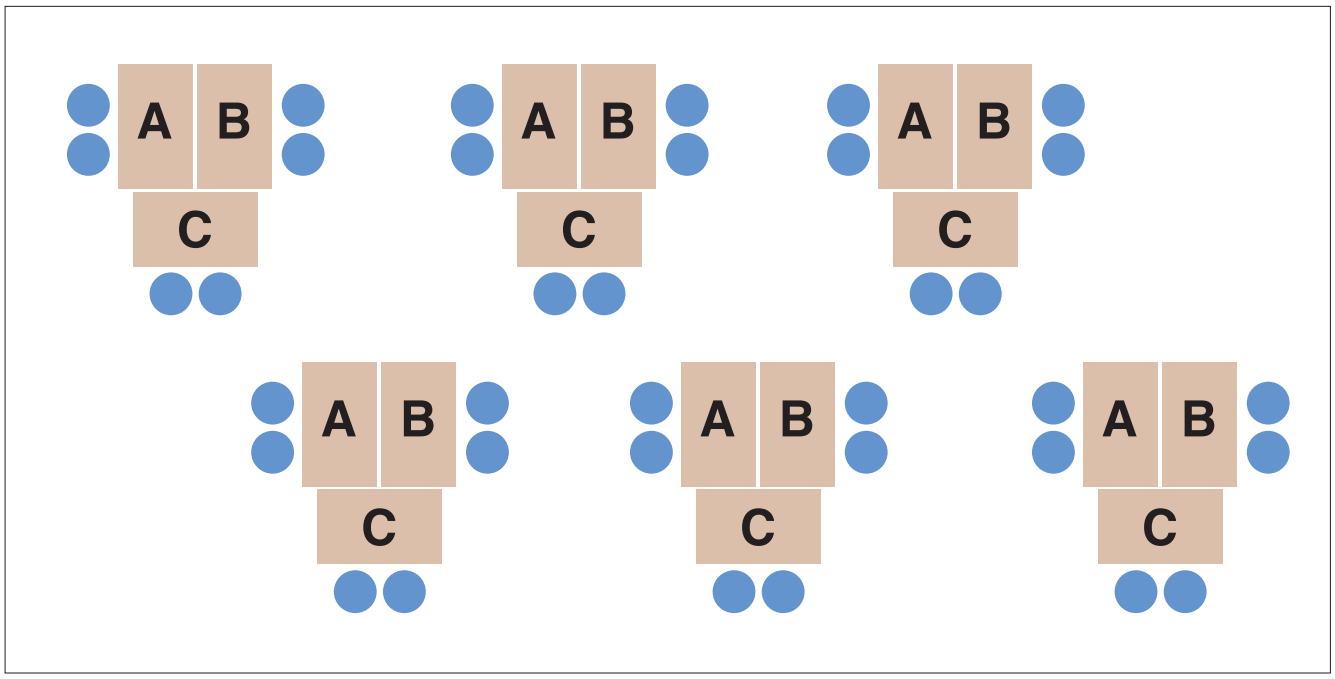



*Figure 1: Example classroom layout; not to scale.*

## LAB ACTIVITY LESSON PLAN

1. Present the Problem Scenario
    a. Have a student read aloud or read together with the class.
    b. Upon completion of reading, assess students understanding of the scenario.
        i. Who are we helping?
        ii. What are we trying to find?
2. Have students move to patient profiles and prediction table
    a. You may also put this up on the projector/screen found in the PowerPoint.
3. Have students work with their partners or groups to discuss and make predictions based on the profiles.
4. Inform students we will now begin our investigation using the ELISA technique.
    a. Remind students what an ELISA is: An assay used to identify the presence of ions or molecules, like antibodies and antigens. We know if something was detected through a color change in the wells.
5. Have students re-label their wells as a refresher for the ELISA investigation.
6. Introduce students on how to use a micropipette
    a. Ask students if they know how to use one. If they say they do, ask a student to come up to the doc cam and demonstrate how to use it while you give out instructions. Otherwise demo how to use micropipette for the students. If no doc cam available, procedures are also in the PowerPoint.
    b. Instructions on how to use a micropipette with doc cam (Students will have a copy of procedures in the student worksheet):
        i. Hold the micropipette in your hand with the fingers under the hook and your thumb on top of the plunger button.
        ii. Explain there are two stops
            1. Demonstrate first stop. First stop is when you push the micropipette plunger down slowly and feel resistance above the top of the micropipette. This first stop will vary based on the volume set on the micropipette.
                a. We use first stop to fill the pipet. This will eject the air out of the pipet tip before it is put into the vials to load up the pipet.
            2. Demonstrate second stop. Second stop is when you push the micropipette plunger all the way down.
                a. We use second stop to eject the contents out of the pipet tip.

iii. Set the micropipette to 50 µL

iv. Take your micropipette and attach a pipet tip to it by pushing shaft of the micropipette onto the pipet tip. Be sure to NOT jam the micropipette shaft into the pipet tip.

v. Take the tube labeled sample (PS)

vi. Go to first stop on the micropipette.

vii. Insert the pipet tip into the liquid and slowly release the plunger.

viii. Transfer 50 µL of the patient sample tube to each well by going to second stop on the micropipette.

ix. Eject the pipet tip into your waste cup by pressing down on the tip ejector found at the top of the micropipette near your thumb placement.

7. There is now a mandatory five-minute wait. Guide students to the antibody matching graphic organizer in their student worksheet. Have students watch the following video. (Also found in the PowerPoint).

8. Instruct students on how to wash the well strips.

    a. Explain to students we waited five minutes before washing the well strips to ensure that the sample serum antigens bound themselves to the sides of the wells.

    b. Make sure you have paper towels laid out in front of you. Identify a dry location on the paper towels that can fit your microplate strip.

    c. Take your microplate strip and tip it upside down onto the dry paper towel location you identified, and GENTLY tap the strip a few times upside down onto the paper towel. We gently tap to avoid having the liquid splash back into the wells and contaminating our work.

    d. Double-check your wells that there is no liquid still inside them.

    e. Place microplate upright in front of you and throw away the wet paper towel and use a new, dry one. OR identify a new, dry location on the paper towel that can fit your microplate strip.

    f. Take your transfer micropipette and set it to 200 µL.

    g. Fill it up with the tube labeled “WB” for washing buffer.

    h. Transfer 200 µL of the washing buffer solution into each well. Be sure not to overfill or spill the solution into the other wells.

    i. Take your microplate strip and tip it upside down on the dry paper towel location you identified earlier and empty out the solution from the wells. You have now done one wash.

    j. Double-check your wells that there is no liquid still inside them.

    k. Repeat the washing process one more time for a total of two washes.

l. Double-check your wells that there is no liquid still inside them.

9. Instruct students to add the primary antibodies. Let them know these are biomarkers to help us identify what type of lung cancer is in the serum.
    a. If students do not know what a biomarker is, let students know they are a way for scientists to measure the healthy, or unhealthy, state of people. Each biological system has their own specific biomarkers.
    b. Make sure students are using a fresh pipet. Confirm why we are using a new pipet.
    c. Take the tube labeled "+". Affirm everyone is on same color tube (ask students)
        i. This tube represents the antibody to identify the biomarker carcino embryonic antigen, or CEA. CEA is a broad biomarker that is present in both types of lung cancer. Adding this antibody into the positive wells will let us detect the presence of any CEA in the serum sample.
    d. Transfer 50 µL of the antibody for the CEA biomarker to the "+" well.
    e. Take the tube labeled "SC". Affirm everyone is on the same color tube.
        i. Ask if we can use the same pipet tip or need a new one.
        ii. This tube represents the antibody for the biomarker pro-gastrin-releasing peptide, or ProGRP. ProGRP is only present in small cell lung cancer. Adding this antibody into the small cell wells will let us detect the presence of any ProGRP in the serum sample.
    f. Transfer 50 µL of the antibody for ProGRP biomarker to the small cell lung cancer well (SC).
    g. Take the tube labeled "NS". Affirm everyone is on the same color tube. Affirm everyone is on a new pipet tip.
        i. This tube represents the antibody for the biomarker neuron-specific enolase, or NSE. NSE is only present in non-small cell lung cancer. Adding this antibody into the non-small cell wells will let us detect the presence of any NSE in the serum sample.
10. Transfer 50 µL of the antibody for NSE biomarker to the non-small cell lung cancer well (NS).
11. There is now another 5-minute mandatory wait period. Guide students to the second antibody matching graphic organizer. Have students watch the following video. (found in the PowerPoint).
12. Have students wash their micro strips twice
13. Add the secondary antibody.
    a. Ask students what they think will happen then if the secondary antibody does not find a primary antibody to bind.
        i. It will lead to no color change, which means it did not detect the biomarker for lung cancer.

b. Make sure students are using a fresh pipet

c. Take the tube labeled “SA”, affirm everyone is on same color tube (ask students).

d. Transfer 50 µL of the secondary antibody to each well.

14. This the last mandatory five-minute wait period. Do the antibody matching game with class.

    a. Explain the rules of the game (found in PowerPoint)

    b. Instructor will call on a single cohort to answer the question by attempting to unlock one of the lock pads with the key they have selected themselves. If correct, students will get a reward. If incorrect, give another group a chance. Make sure all cohorts get at least one chance to answer.

    c. ALTERNATIVE: The instructor may lock all the locks together and students would need to unlock all of them in a certain number of tries to receive a class reward. Rewards could be points towards a pizza party, bonus points on next quiz/test, receive full points on one missed homework assignment, etc.

15. Have students wash their micro strips twice

16. Add the enzyme substrate.

    a. Make sure students are using a fresh pipet

    b. Take the tube labeled “SUB”.

    c. Transfer 50 µL of the enzyme substrate to each well.

    d. This may take up to five minutes.

    e. If not done with the antibody matching game, continue here as students wait for the color change to occur in the ELISA.

17. Guide students to data collection and analysis.

18. Inform students to draw in their results in the diagram and properly label.

19. Have students revisit their predictions and fill in the prediction table and determine if they were correct or incorrect.

20. Have students complete a CER table for each patient serum.

21. Have the class report out the results using their CER table.

    a. SAMPLE: “Our cohort believes patient A has small cell lung cancer, because of the symptoms for patient A were x,y,z. Patient A also had the wells turn blue for both the positive and small cell cancer wells. Based on the data collected, there was a high expression of the CEA and ProGRP biomarker suggesting high chance of small cell lung cancer.”

22. If there is time available, discuss the idea of a false positive.
    a. What is it? How could it happen?
23. Talk to students about two STEM careers related to this activity, see slide 18.

# STUDENT LAB WORKSHEET (TEACHER'S KEY)

## ELISA Lung Cancer Student Lab Worksheet

Students will be taking on the role of a lab technician at a biomedical research facility to assist a doctor in the state of Maryland in handling patient samples confirmed for lung cancer. Students will receive patient profiles to go along with the patient samples and will be making predictions as to what type of lung cancer each patient has. They will perform an ELISA on the samples and then confirm their predictions.

## Problem Scenario

Dr. Bryant is a doctor at the Discovery Clinic. The clinic is located in one of the many hot zones for radon in the state of Maryland. Some of her older patients have started to demonstrate some symptoms associated with lung disease and she has used computed tomography (CT) scans to confirm that they have lung cancer. She has sent over serum samples from three different patients to your laboratory to determine which type of lung cancer the patients have, so she can propose treatment. She knows that your biomedical research facility has the tools readily available for a quick turnaround.

On the next page are the patient profiles that Dr. Bryant has sent over. With your team members, use the prediction table to determine which type of lung cancer each patient has and what characteristics or risk factors led you to those predictions.

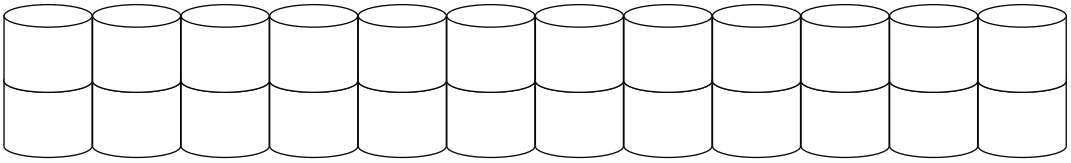
## PATIENT PROFILES KEY

| | |
|---|---|
|  <br>Sample A (Small Cell) | • Male<br>• Non-smoker, heavy secondhand smoke from partner<br>• Coughing up blood |
|  <br>Sample B (Non-Small Cell) | • Female<br>• 2 packs per day (ppd) for 10+ years<br>• Chest Pain<br>• Family history of lung cancer |
|  <br>Sample C (False Positive) | • Female<br>• Non-smoker<br>• Chronic cough |

### Prediction Table Key

| Patient Profile Prediction Table | | |
|---|---|---|
| Patient Profile | Characteristics/Risk Factors Associated with Lung Cancer | Prediction (Small cell/ Non-small cell) |
| A | | Small cell |
| B | | Non-Small cell |
| C | | False Positive |

**Now that you have completed your predictions, we will begin our investigation using ELISA. First, we will need to label our wells in the diagram below to make sure we understand what we problem we are solving.**



**Now we will review how to use the tools needed for a successful ELISA and add our serum samples to the wells in the process.**

## MICROPIPETTE INSTRUCTIONS

1. Hold the micropipette in your hand with the fingers under the hook and your thumb on top of the plunger button.
2. There are two stops
    a. First stop is when you push the micropipette plunger down slowly and feel resistance above the top of the micropipette. This first stop will vary based on the volume set on the micropipette.
        i. We use first stop to fill the pipet. This will eject the air out of the pipet tip before it is put into the liquid to load the pipet.
    b. Second stop is when you push the micropipette plunger all the way down.
        i. We use second stop to eject the contents out of the pipet tip.
3. Set the micropipette to 50 µL
4. Take your micropipette and attach a pipet tip to it by pushing shaft of the micropipette onto the pipet tip. Be sure to NOT jam the micropipette shaft into the pipet tip.
5. Take the tube labeled sample (PS)
6. Go to first stop on the micropipette.
7. Insert the pipet tip into the liquid and slowly release the plunger.
8. Transfer 50 µL of the patient sample tube to each well by going to second stop on the micropipette.
9. Eject the pipet tip into your waste container by pressing down on the tip ejector found at the top of the micropipette near your thumb placement.

We now need to wait for the serum sample antigens to bind onto the wells. In the meantime, watch the video on antibodies and match the descriptions on the table below:

## ANTIBODY MATCHING GAME PART 1 (ANSWER KEY)

| Lock | Key |
|---|---|
| 1. What is an antigen? | a. White blood cell |
| 2. What is the shape of antibodies? | b. Identify antigens |
| 3. What is an antibody? | c. Y-shaped |
| 4. What is the function of antibodies | d. Neutralize antigens |
| | e. B cells |
| | f. Foreign substance in the body that triggers an immune response |
| | g. C-shaped |
| | h. Protein produced by your immune system |

Our serum samples have had enough time to bind onto the walls of the wells. We will now wash the wells out before moving onto the next step of ELISA.

### Washing microplate strip instructions

1. Make sure you have paper towels laid out in front of you. Identify a dry location on the paper towels that can fit your microplate strip.
2. Take your microplate strip and tip it upside down onto the dry paper towel location you identified, and GENTLY tap the strip a few times upside down onto the paper towel. We gently tap to avoid having the liquid splash back into the wells and contaminating our work.
3. Double-check your wells that there is no liquid still inside them.
4. Place microplate upright in front of you and throw away the wet paper towel and use a new, dry one. OR identify a new, dry location on the paper towel that can fit your microplate strip.
5. Take your transfer micropipette and set it to 200 µL.
6. Fill it up with the tube labeled “WB” for washing buffer.
7. Transfer 200 µL of the washing buffer solution into each well. Be sure not to overfill or spill the solution into the other wells.
8. Take your microplate strip and tip it upside down on the dry paper towel location you identified earlier and empty out the solution from the wells. You have now done one wash.
9. Double-check your wells that there is no liquid still inside them.

10. Repeat the washing process one more time for a total of two washes.
11. Double-check your wells that there is no liquid still inside them.
12. With our wells fully washed, we can now go ahead and add the primary antibodies to the wells. This will help us identify unique biomarkers found in lung cancer.

1. Place a fresh pipet tip onto your micropipette.
2. Take the tube labeled “+”.
    a. This tube represents the antibody to identify the biomarker carcino embryonic antigen, or CEA. CEA is a broad biomarker that is present in both types of lung cancer. Adding this antibody into the positive wells will let us detect the presence of any CEA in the serum sample.
3. Transfer 50 µL of the antibody for the CEA biomarker to the “+” well.
4. Take the tube labeled “SC”.
    a. This tube represents the antibody for the biomarker pro-gastrin-releasing peptide, or ProGRP. ProGRP is only present in small cell lung cancer. Adding this antibody into the small cell wells will let us detect the presence of any ProGRP in the serum sample.
5. Transfer 50 µL of the antibody for ProGRP biomarker to the small cell lung cancer well (SC).
6. Take the tube labeled “NS”.
    a. This tube represents the antibody for the biomarker neuron-specific enolase, or NSE. NSE is only present in non-small cell lung cancer. Adding this antibody into the non-small cell wells will let us detect the presence of any NSE in the serum sample.
7. Transfer 50 µL of the antibody for NSE biomarker to the non-small cell lung cancer well (NS).

Just like before, we now need to wait for the antibodies to bind onto the antigens in the well. So, watch this video on monoclonal antibodies and match the descriptions on the table below:

## Antibody Matching Game Part 2 (ANSWER KEY)

| Lock | Key |
|---|---|
| 5. How many antigens can antibodies target? | i. Two |
| 6. What is a monoclonal antibody? | j. Unknown process |
| 7. What is the function of monoclonal antibodies? | k. Expensive |
| 8. Why can’t we use monoclonal antibodies more often? | l. Develop vaccinations |
| | m. Targeting specific antigens |
| | n. Animal antibody tested on humans |
| | o. Cloned antibody produced in a lab |
| | p. One |

We will once again wash out the wells twice.

## Washing microplate strip instructions

1. Make sure you have paper towels laid out in front of you. Identify a dry location on the paper towels that can fit your microplate strip.
2. Take your microplate strip and tip it upside down onto the dry paper towel location you identified, and GENTLY tap the strip a few times upside down onto the paper towel. We gently tap to avoid having the liquid splash back into the wells and contaminating our work.
3. Double-check your wells that there is no liquid still inside them.
4. Place microplate upright in front of you and throw away the wet paper towel and use a new, dry one. OR identify a new, dry location on the paper towel that can fit your microplate strip.
5. Take your transfer micropipette and set it to 200 µL.
6. Fill it up with the tube labeled "WB" for washing buffer.
7. Transfer 200 µL of the washing buffer solution into each well. Be sure not to overfill or spill the solution into the other wells.
8. Take your microplate strip and tip it upside down on the dry paper towel location you identified earlier and empty out the solution from the wells. You have now done one wash.
9. Double-check your wells that there is no liquid still inside them.
10. Repeat the washing process one more time for a total of two washes.
11. Double-check your wells that there is no liquid still inside them.

Now we can add the secondary antibodies. These antibodies will bind onto the primary antibody from the previous step.

## ELISA INVESTIGATION
## ADDING THE SECONDARY ANTIBODIES

1. Make sure you are using a fresh pipet tip.
2. Take the tube labeled "SA".
3. Transfer 50 µL of the secondary antibody to each well.

While we wait for the secondary antibodies to bind onto the primary antibodies, we're going to use those description tables for this next game. The rules will be explained by your teacher and on the PowerPoint.

Our five minutes are up, hopefully you were able to unlock all the locks! If not, there is still some time available after this next step. We're going to once again wash out the microplate wells. If you need a reminder on how to wash the well strips, they can be found at the top of this page.

Now we will add the enzyme substrate. This will tell us whether it detected any of the biomarkers for lung cancer by changing the color of the wells.

ELISA INVESTIGATION

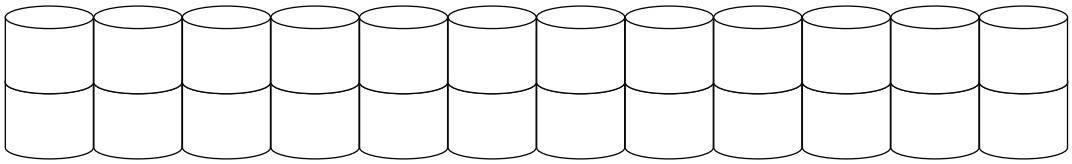
# ADDING THE SUBSTRATE

1. Make sure students are using a fresh pipet
2. Take the tube labeled “SUB”.
3. Transfer 50 µL of the enzyme substrate to each well.

If you haven't finished your lock and key game, go ahead and finish it while we wait for the results. If completed, you may have to wait up to five minutes to see the results for your ELISA. You will slowly see a change in color over time if the biomarkers are detected.

## DATA COLLECTION AND ANALYSIS

1. Go report out your results onto the wells diagram below. Shade in the wells to represent the expression of biomarker presence detected. Remember to label it.



2. In your cohort, go back and look at your predictions on the student worksheet. Were your predictions correct? Which ones were incorrect?

| Patient Profile Prediction Table | | | |
|---|---|---|---|
| Patient Sample Profile | Prediction | Result | Correct/ Incorrect |
| A | | | |
| B | | | |
| C | | | |

3. On the following pages: Using the data you have available, use the claim, evidence, reasoning table to make your conclusion about each patient sample.
    a. Sample answers have been provided below. Student handout will have sentence starters for Sample A.

## CER TABLE Sample A

| Claim, Evidence, Reasoning Table | |
|---|---|
| Claim | "My patient has small cell lung cancer." |
| Evidence | "My patient's symptoms included a, b, c, and the associated risk of x. Their sample also turned blue in the small cell cancer well, which means positive." |
| Reasoning | "Based on the data collected, there was a high expression of the CEA and ProGRP biomarker found in the patient's serum sample." |

## CER TABLE Sample B

| Claim, Evidence, Reasoning Table | |
|---|---|
| Claim | "My patient has non-small cell lung cancer." |
| Evidence | "My patient's symptoms included a, b, c, and the associated risk of x. Their sample also turned blue in the non-small cell cancer well, which means positive." |
| Reasoning | "Based on the data collected, there was a high expression of the CEA and NSE biomarker found in the patient's serum sample." |

## CER TABLE Sample C

| Claim, Evidence, Reasoning Table | |
|---|---|
| Claim | "My patient has no lung cancer." |
| Evidence | "My patient's symptoms included a, b, and c, but had no major risk factors. Their sample did not change color in any of the wells." |
| Reasoning | "Based on the data collected, there was a low expression of CEA, NSE, and ProGRP biomarkers found in the patient's serum sample." |

Activity was developed based on data from the following paper:

Zamay, T., Zamay, G., Kolovskaya, O., Zukov, R., Petrova, M., Gargaun, A., Berezovski, M. and Kichkailo, A., 2017. Current and Prospective Protein Biomarkers of Lung Cancer. Cancers, 9(12), p.155.