FUTURELAB+

BIOMED

Taking Action in Your Community: Health Equity

Using ELISA in Vaccine Trials

Laboratory Investigation

Developed in partnership with:

Bay Area Bioscience Education Community

In this Lesson Plan:

Print the **Teacher Section** →

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Teachers [T] *and* Student Resources [S] *can be printed independently. Select the appropriate printer icon above to print either section in its entirety.*

Follow the tips below in the Range field of your Print panel to print single or a range of pages:

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Cover Image
This is an illustration of coronavirus particles.

BIOMED / TAKING ACTION IN YOUR COMMUNITY: HEALTH EQUITY

Lab: Using ELISA in Vaccine Trials

DRIVING QUESTION

Could results obtained from an ELISA for IgG antibodies provide evidence that a COVID-19 vaccine works?

ACTIVITY DURATION

5 class sessions
(45 minutes each)

OVERVIEW

Vaccines have revolutionized human health by effectively eradicating deadly diseases, such as smallpox, measles, mumps and polio. Today, as the global population grapples with the COVID-19 pandemic, vaccinations will play a critical role in controlling the spread of this disease. Vaccine development and testing is typically a long, complex process that involves standard steps. The first stages involve research and animal testing, while the latter stages include clinical studies with human subjects and regulatory oversight.

Efficacy is determined in these clinical trials by comparing a group of people who got the vaccine to a group of people who did not get the vaccine, and seeing how many cases of the disease are in one group compared to the other. *Effectiveness* is a term that refers to what happens in the real world, outside of a controlled environment, again by comparing the reduction of disease between vaccinated and unvaccinated groups of people. Researchers today are working to determine the correlate of protection between antibody concentration and protection from COVID-19. If successful, they would no longer have to wait for trial volunteers to get the disease, as they do now.

In this lab, students will be conducting research on an approved COVID-19 vaccine to identify the level of antibodies that confers immunity. Working in pairs, students will determine the concentration of IgG antibodies against SARS-CoV-2 virus in 6 clinical trial participant samples one month post vaccination. They will be using a mock ELISA test to measure this concentration of antibodies in their participant samples (this lab uses BSA protein and Lowry reagent to simulate ELISA results).

ESSENTIAL QUESTIONS

How can we use an investigation to obtain evidence to determine if a vaccine protects someone from a disease?

How can an ELISA be used in an investigation to determine an individual's response to a vaccine?

How do the structure of antibodies help them function in the fight against infection?

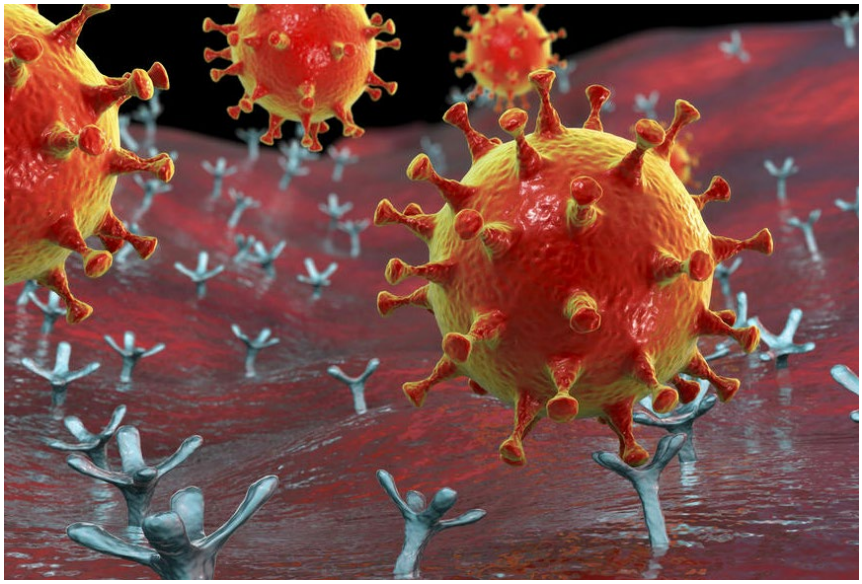
BACKGROUND INFORMATION

Proficiency in micropipetting and serial dilution technique are skills necessary for this lesson. This lesson includes 2 stand-alone days' worth of reference materials and opportunities for student practice that can be helpful to implement at any point in the year before this lesson. Familiarity with the anatomy and physiology of the immune system is important to understanding how vaccines work. At this point in the unit, students will have already been introduced to epidemiology, pathogens, how vaccines work and the vaccine development process. Familiarity with solving linear equations ($y = mx + b$) and creating graphs using spreadsheet software is helpful for the "standard curve" portion of the lesson.

Have you ever wondered…

What happens in our bodies when we get a vaccine?

Vaccines have allowed us to prevent diseases that once threatened many lives. A vaccine works by introducing a weakened or killed version of the disease-causing pathogen or some component of the pathogen (e.g. antigens or DNA/RNA) into the body. This trains our immune system to create antibodies and memory cells that fight the disease, just as would happen when we are exposed to a disease but—crucially—vaccines work without making us sick.



MAKE CONNECTIONS!

How does this connect to the larger unit storyline?

Vaccines work by priming the immune response i.e. memory T and B cells, and antibody production to defend the body against a pathogen. We can potentially measure antibody concentration as a measure of vaccine efficacy.

How does this connect to careers?

Vaccine research scientists study how the body's immune system works, how it can prevent disease, and what type of developments in medicine could help lead to the prevention of disease. They may plan, lead, and carry out investigations to determine whether a vaccine is working.

How does this relate to the product development life cycle?

After developing a new vaccine, further monitoring and research is conducted to determine vaccine effectiveness.

Pedagogical Framing

Instructional materials are designed to meet national education and industry standards to focus on in-demand skills needed across the full product development life cycle—from molecule to medicine—which will also expose students and educators to the breadth of education and career pathways across biotechnology.

Through this collection, educators are equipped with strategies to engage students from diverse racial, ethnic, and cultural groups, providing them with quality, equitable, and liberating educational experiences that validate and affirm student identity.

Units are designed to be problem-based and focus on workforce skill development to empower students with the knowledge and tools to be the change in reducing health disparities in communities.

SOCIAL-EMOTIONAL LEARNING

This lab engages students in effective communication and collaborative problem-solving as they gain proficiency in core lab techniques, such as micropipetting, serial dilutions, and ELISA. After students perform the experiment, there is an opportunity to make a reasoned judgment by analyzing information, data, and facts acquired throughout the lesson.

CULTURALLY AND LINGUISTICALLY RESPONSIVE INSTRUCTION

This lesson is centered on the COVID-19 pandemic and the associated vaccines being produced, which grounds the lesson in current, real-world events. Additionally, most, if not all, students have first-hand experience getting a vaccine. There may be opportunities to discuss ethical issues related to vaccines, such as mandated immunizations, research and testing (i.e. who is included in the trials), consent and access.

COMPUTATIONAL THINKING PRACTICES

Students will engage in finding patterns, collecting and analyzing data throughout this lesson. Students identify patterns as they prepare their serial dilution for the standard curve and relate the color signal readings to IgG concentrations. Students will collect color signal data for their participant samples and use a mathematical equation to calculate IgG concentrations. Analyzing this data will allow students to determine if a particular level of antibodies confers immunity to COVID-19. Lastly, they will analyze class data, looking for patterns that reveal additional information related to the driving question.

OBJECTIVES

Students will be able to:

Obtain and **communicate** information as they construct explanations about how vaccines trigger an immune response and how the production of antibodies protects against future infection.

Collect data using ELISA to create a standard curve and use that standard curve to **analyze** and **interpret** data about the level of antibodies in vaccine clinical trial participant blood samples.

Support an argument regarding the correlate of protection for COVID-19 vaccines with evidence from data collected from their experimentation.

Materials

Documents

- **Preparing the Classroom for the Lab (for teacher)**
- **Background Reading: Vaccines and the Immune System (1 per student)**
- **Background Reading: ELISA (1 per student)**
- **Vocabulary Tool (1 per student)**
- **Student Guide (1 per student)**
- **Student Protocol, Part 1: Serial Dilution and ELISA (1 per pair)**
- **Student Protocol, Part 2: Color Signal and Standard Curve (1 per pair)**
- **96-well Plate Template (1 per pair)**

For "Building Lab Skills" Stand-Alone days

- **Building Lab Skills: Micropipetting Reference Guide and Practice (1 per student)**
- **Precision Pipetting Practice Cards (1 set per student)**
- **Optional: Micropipetting Practice: ROY GEE BIV (1 per student)**
- **Building Lab Skills: Serial Dilutions and the Standard Curve (1 per student)**

Lab Equipment and Reagents

- **Waste bucket (1 per pair)**
- **P20 micropipette (1 per pair)**
- **P200 micropipette (1 per pair)**
- **Micropipette tips (1 per pair)**
- **Microtube rack (1 per pair)**
- **96-well plate (1 per pair)**
- **96-well template card (1 per pair)**
- **6 empty 1.5-mL tubes (1 per pair)**
- **Water (1 per 2 pairs)**
- **Standards stock (1 µg/mL) (1 per 2 pairs)**
- **Blood plasma antibody (1 per 2 pairs)**
- **Spike protein antigen (1 per 2 pairs)**
- **Substrate (1 per 2 pairs)**
- **Labeled antibody (1 per 2 pairs)**
- **Participant samples a–f (1 per 2 pairs)**
- **Permanent marker (1 per pair)**
- **Gloves (optional) (1 per pair)**

Other

- **Smartphone with a free microplate reader e.g. Spotxel app (1 per pair)**
- **Computer with spreadsheet software (1 per student)**

Skills

Procedure

Building Lab Skills 1

Micropipetting

Teacher Note > *There are 2 stand-alone days that focus on building lab skills (1 for Micropipetting and 1 for Serial Dilution) in which students must be proficient before completing the 5-day "Using ELISA in Vaccine Trials" lab.*

LEARNING OUTCOMES

Students will be able to:

Demonstrate proper use of micropipettes by accurately and precisely measuring small volumes (in the metric unit micro-liters) using protocols.

Select the appropriate micropipette, accurately read and set the dial using protocols.

Whole Group (20 minutes)	
1	Prior to students arriving: Make a class set of the *Precision Pipetting Practice Cards* and put each card in a sheet protector or laminate for reuse.
2	Prompt students with the following warm-up question for discussion: Have you seen a micropipette before? It may be helpful to show a short video clip of a micropipette being used, such as in a forensics lab or during vaccine development.
3	Explain that today's lesson will focus on this essential piece of biotechnology lab equipment, which will enable students to perform further experiments.
4	Pass out micropipettes to student pairs and have them make observations and ask questions about the micropipettes. Encourage students to notice similarities and differences.
5	Pass out copies of the *Building Lab Skills: Micropipetting Reference Guide* to each student. Read the 'Key Vocabulary' and *Parts of a Micropipette* together as a class, taking care to check for student understanding of the units, microliters.
6	Model the mechanics of how to use a micropipette while having students read and annotate the *Micropipetting Instructions*. Ask students to practice and show their partner their technique for feedback until they feel confident. Optional: Show University of Leicester, *Using a Micropipette* video.
7	Explain the difference between the P20, P200 and P2000 micropipettes using the information in the table *Choosing a Micropipette*.

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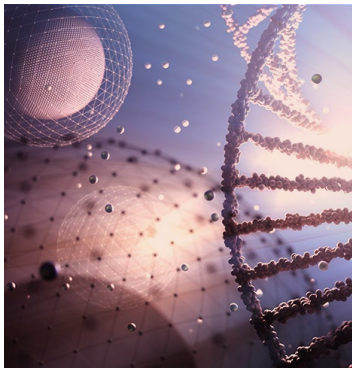
Skills

Continued



Procedure

Small Group (20 minutes)	
1	Pass out copies of *Building Lab Skills: Micropipetting Practice* to each student. Ask students to fill in the blanks in #1 and #2 to practice reading the volume of a given micropipette and dial setting. It may be helpful to do the first one as an example and review.
2	Pass out *Precision Pipetting Practice Cards* and the colored liquid. Explain that now students will practice using the P20 and P200 micropipettes to transfer precise, accurate volumes onto a waterproof card.
3	Students can compare their droplet sizes to other classmates or you may wish to check by circulating with your own micropipette and sucking up their droplets.

Individual (5 minutes)	
1	Reflection/Exit Ticket: *What do you think are the 3 most important things to keep in mind while using a micropipette?*

Optional Extension: Micropiptetting Practice ROY GEE BIV (30 minutes)	
1	Ask students to read the *Background* and *Directions* section of the *Micropiptetting Practice ROY GEE BIV*.
2	Have students complete #1 and #2 by filling out the *Procedure Table* and expected final volumes in the *Reaction Tube table* and review as a class.
3	Break students into pairs, pass out lab materials, and instruct students to follow the rest of the directions and answer the questions. Encourage students to continue to provide each other with feedback on their technique and proper use of the micropipettes.

Skills

Continued

Procedure

Building Lab Skills 2

Serial Dilution and Standard Curve

Teacher Note > *This lesson focuses on the fundamental skill of serial dilution and thus can be completed as a stand-alone lesson any point before the five-day "Using ELISA in Vaccine Trials" lab.*

LEARNING OUTCOMES

Students will be able to:

Perform a serial dilution using the Standard Stock solution.

Describe the purpose of a standard curve, how to make one, and how to use one in an investigation to determine an unknown concentration of a substance in a sample.

Prepare in Advance	
1	Prepare a stock solution of water with food coloring of any color (the darker, the better) and aliquot into small containers of ~3mL or more per pair of students.
2	Set up the following materials per pair: • P1000 micropipette and tips • 4 test tubes (ex. Conical tubes) or small containers (10–50mL) • 1 graduated cylinder (10–50mL) • Labeling tape • Permanent marker

Whole Group (10 minutes)	
1	Warm-up: Describe how and why you would want to dilute a solution in your everyday life.
2	Share with students that they will be using micropipettes to practice a new lab skill called *serial dilution* and learn how serial dilutions are used to make standard curves.

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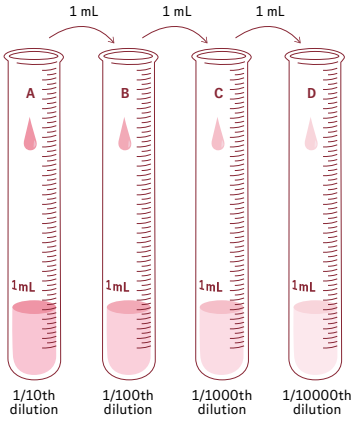
Skills

Continued



This graphic illustrates serial dilution.

Procedure

Small Group (20 minutes)

1	Pass out *Background Reading and Practice: Serial Dilution and the Standard Curve* and lab materials.
2	Ask students to perform the *Hands-on Practice* section with their partners and then complete the *Serial Dilutions* section of the reading.
3	Ask students to answer question #1. They should check their answers with the key.
4	Ask students to clean up by pouring their solutions down the drain and discarding the tube labels in the trash.

Small Group (10 minutes)

1	Ask students to work with their partners to complete the *Standard Curve* section of the reading and answer questions #2–5. They should check their answers with the key.

Individual (5 minutes)

1	Reflection/Exit Ticket: Create a model that illustrates what a serial dilution is on a molecular level (use a symbol to represent a molecule).

Day 1

LEARNING OUTCOMES

Students will be able to:

Describe how vaccines elicit the immune response and the production of antibodies, using scientific text.

Construct an explanation of how an indirect ELISA is used to detect the amount of a certain protein in a sample using scientific text.

Procedure

Whole Group (10 minutes)

1. Warm up with *Stand and Share*: What do you know about vaccines? How do we know a vaccine works?

2. Show the following videos and ask students to record evidence from each video that supports their answers to the warm-up:
   - mRNA Vaccine video, UCSF: *How do the new mRNA vaccines for COVID-19 work?*
   - ELISA video, University of Michigan: *Enzyme-Linked Immunosorbent Assay (ELISA)*

3. Ask students to share the evidence and ideas they record with a partner.

Small Group (30 minutes)

1. Ask students to complete *Background Reading: Vaccines and the Immune System* and check their answers with the key.

2. Ask students to complete *Background Reading: ELISA (Enzyme-linked Immunosorbent Assay)* and check their answers with the key.

3. You may wish to have student pairs *jigsaw* these readings by focusing on one and then teaching their partner about their reading. Students should still individually understand the content and complete the questions.

Individual (5 minutes)

1. Reflection/Exit Ticket: List any lingering questions you have about vaccines or ELISA.

2. Ask students to write their own sentence for each word in the *Vocabulary Tool* for homework.

Day 2

LEARNING OUTCOMES

Students will be able to:

Predict if there will be IgG antibodies in the participant blood samples using information about clinical trial volunteers.

Obtain and **communicate** information about the variables and components of their lab using scientific text.

Procedure

Whole Group (10 minutes)	
1	Warm up with *Stand and Share:* What is the purpose of an ELISA? Considering what you know about vaccines, why might someone want to perform an ELISA?
2	Address student questions from the previous session student Exit Tickets.
3	Pass out the *Student Protocol, Part 1: Serial Dilution and ELISA* and *Student Guide*.
4	Read the context provided in *Student Guide, Part 1: Pre-Lab* together as a class, taking time to make annotations that increase reading comprehension. Make sure to address any questions that come up for students.

Small Group (30 minutes)	
1	With their lab partners, have students complete *Student Guide, Part 1: Pre-Lab*. Circulate and monitor for comprehension. You may wish to stamp correct responses as a 'ticket' to the lab for the next day.
2	Early finishers can read and annotate their *Student Protocol* so they are ready to perform the lab. You may also wish to have students download a free microplate reader app such as *Spotxel,* on their smartphones in preparation for Day 4.

Individual (5 minutes)	
1	Exit Ticket: What is the purpose of adding *Substrate* in the ELISA? Have students share their written responses with a *Turn and Talk.*

Day 3

LEARNING OUTCOMES

Students will be able to:

Perform a serial dilution and a mock indirect ELISA to determine the concentration of each participant's IgG antibodies to SARS-CoV-2 spike protein after vaccination using protocols.

Procedure

Teacher Note > *In order to complete this lab, students must first become familiar with micropipetting and serial dilution techniques (See Building Lab Skills). Before students arrive, use the Lab Preparation Doc to aliquot reagents and set up one lab station for every two pairs of students. Note that students will be using a mock ELISA test, which uses BSA protein and Lowry reagent instead of antibodies*

Whole Group (5 minutes)	
1	Introduce the lab to students and inform them that they will be working in pairs to determine the concentration of IgG antibodies against SARS-CoV-2 virus in 6 clinical trial participant samples after they were vaccinated one month earlier.

Small Group (35 minutes)	
1	Ask students to complete the instructions in *Student Protocol, Part 1: Serial Dilution and ELISA.*
2	When students have finished their testing, they will follow the clean-up procedures listed.

Individual (5 minutes)	
1	Exit Ticket: On a scale of 1–5 (1 being lowest and 5 being highest), how confident are you that you set up the ELISA correctly? Give at least two pieces of evidence for your rating.

Teacher Note > *If you have a longer class period, you may wish to have students incubate their plates for 30 minutes after completing the Student Protocol, Part 1: Serial Dilution and ELISA and then continue with Student Protocol, Part 2: Color Signal.*

Day 4

Procedure

LEARNING OUTCOMES

Students will be able to:

Perform an investigation using ELISA technique to collect, analyze, and interpret color signal data and explain observations from an experiment using protocols and a smartphone app.

Apply their knowledge of serial dilutions to create a standard curve graph using protocols and spreadsheet software.

Apply their knowledge of standard curves to determine the concentration of antibodies in vaccine clinical trial participant blood samples using a linear trendline equation.

Whole Group (10 minutes)	
1	Ask one student from each pair to pick up their 96-well plate from the previous day and the *Student Protocol, Part 2: Color Signal.*
2	Warm-up: Write out step by step how to solve for "y" in a linear equation $(y = mx + b)$.
3	Ask a student with the correct answer to explain it to the class. (This is a great opportunity to invite a student who rarely shares out. Make sure you check the student has the correct answer and is will to share out first.)
4	Share with students that in this session they will measure the color signal from their ELISA, use the data from the serial dilution to create a standard curve, and use the standard curve to calculate the concentration of IgG antibodies in vaccine clinical trial participant blood samples.

Small Group (10 minutes)	
1	Ask students to complete the *Observations/Explanations* table on the *Student Guide Part 2* with their lab partners.
2	Ask one student from each pair to download the plate reader app on a smartphone and complete the *Student Protocol, Part 2: Color Signal* together. Once students have a screenshot of the 96-well plate with color signals, they should record the data in the table on the *Student Guide, Part 2: Lab*.

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Day 4

Continued

Procedure

Individual (15 minutes)

1 Each student should complete the *Student Protocol, Part 2: Standard Curve* individually, even though they are sharing color signal data with their partners.

2 Ask each student to compare the equations of his or her graph with the partner's graph. Since they used the same data, the graphs should be the same. If they are different, have them repeat the graphing protocol to correct the error.

Small Group (10 minutes)

1 Ask students to complete questions #1–3 on the *Student Guide Part 3: Data Analysis* with their lab partners and finish for homework.

Day 5

Procedure

LEARNING OUTCOMES

Students will be able to:

Apply their knowledge of ELISA technique to identify patterns in antibody concentration using data collected from an investigation.

Write a claim regarding the correlate of protection for COVID-19 vaccines and support it with evidence and reasoning using data collected from an investigation.

Whole Group (5 minutes)

1 Collect class data for participant IgG concentrations on a spreadsheet or whiteboard (1 set of data per pair). Calculate class averages. Option: include the "color signal" data, which are likely to have very different numbers due to differences in lighting, phone cameras, etc. This can lead to interesting discussion.

	Participant IgG Concentration (µg/mL)					
	a	b	c	d	e	f
Group 1						
Group 2						
Group 3						
Average						

Small Group (20 minutes)

1 Ask students to complete questions #4–5 on the *Student Guide Part 3: Data Analysis* and questions #1–4 *Part 4: Arguing from Evidence* with their partners.

Whole Group (5 minutes)

1 Divide the class into groups of three to four lab pairs and ask them to form a circle with their group.

2 Ask each lab pair to take turns presenting their answers to questions #3 of *Part 4: Arguing from Evidence*.

3 Ask each pair to share a piece of evidence they heard from another pair that was particularly compelling and explain why.

Individual (5 minutes)

1 Ask students to individually begin writing a CER paragraph that answers the driving question in *Part 4: Arguing from Evidence* #5 and complete it as homework.

National Standards

Next Generation Science Standards

LS1.A: Structure and Function
Systems of specialized cells within organisms help them perform the essential functions of life.

Analyzing and Interpreting Data
Apply concepts of statistics and probability (including determining function fits to data, slope, intercept, and correlation coefficient for linear fits) to scientific and engineering questions and problems, using digital tools when feasible.

Engaging in Argument from Evidence
Make and defend a claim using evidence from an investigation and scientific reasoning.

Scale, Proportion, and Quantity
Using the concept of orders of magnitude.

Science is a Human Endeavor
Science and engineering are influenced by society and society is influenced by science
and engineering.

Cause and Effect
Empirical evidence is required to differentiate between cause and correlation and make claims about specific causes and effects.

Next Generation Science Standards

Math

MP.2 Reason abstractly and quantitatively.
Perform serial dilutions to solutions of varying concentrations.

MP.4 Model with mathematics.
Use a standard curve to determine unknown concentrations of solutions.

MP.5 Use appropriate tools strategically.
Create a standard curve using a spreadsheet.

Standards for Mathematical Practice | Common Core State Standards Initiative

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National Standards

Continued

Career and Technical Education (CTE)	**A3.4** Employ standard protein techniques, including antibody production, enzyme assays, spectrophotometry, gel electrophoresis, and chromatography and document and evaluate results.
	A4.7 Condict indicator tests for the common macromolecules of the cell.
	A6.1 Apply knowledge of symbols, algebra, and statistics to graphical data presentation.
	A6.3 Calculate and prepare solutions of various molarity; calculate and prepare buffers of various pH; and prepare serial dilutions.
	A6.4 Create data tables and graphs using Excel for the purpose of collecting and analyzing data.
	A8.1 Follow written protocols and oral directions to perform a variety of laboratory and technical tasks.
	A8.6 Properly and safely use and monitor a variety of scientific equipment, including pH meters, microscopes, spectrophotometers, pipettes, micropipettes, and balances.
	A8.7 Determine which equipment is appropriate to use for a given task and the units of measurement used.

Lab

Preparation

KEY

When the preparation task should take place in relationship to the lab

The amount of time necessary to complete the preparation task

Quick Tips

1	Before continuing, check the *Materials List* to make sure you have all the necessary equipment and reagents for the lab. This is a mock ELISA that uses a Lowry protein assay technique to simulate antibody results.
2	We recommend having students complete this lab in **pairs**.
3	*Virtual Learning Options*, including digital-only resources, are provided.

Preparation

1 Before the lab — 1–2 hrs

☐ Prep the lab

— Aliquot the reagents below.
— The directions below are aliquots for 2 pairs of students to share so that prep work can be minimized.
— Participant samples a–f are the "blood plasma".

Tip > *Use different colored tubes to keep each reagent separate.*

	Label on Tubes	Volume Aliquots per pair	Actual Component
1	Standard Stock (1 µg/mL)	425 µL	BSA (2 mg/mL)
2	Antigen (Spike protein)	2 tubes of 1.25 mL each	Lowry reagent
3	Labeled Antibody	250 µL	Water
4	Substrate	200 µL	FC reagent
5	Water	1.1 mL	Water
6	Participant a	125 µL	BSA (1.2 mg/mL)
7	Participant b	125 µL	BSA (0.4 mg/mL)
8	Participant c	125 µL	BSA (1.8 mg/mL)
9	Participant d	125 µL	BSA (0.4 mg/mL)
10	Participant e	125 µL	BSA (1.2 mg/mL)
11	Participant f	125 µL	BSA (1.8 mg/mL)

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Lab

Continued

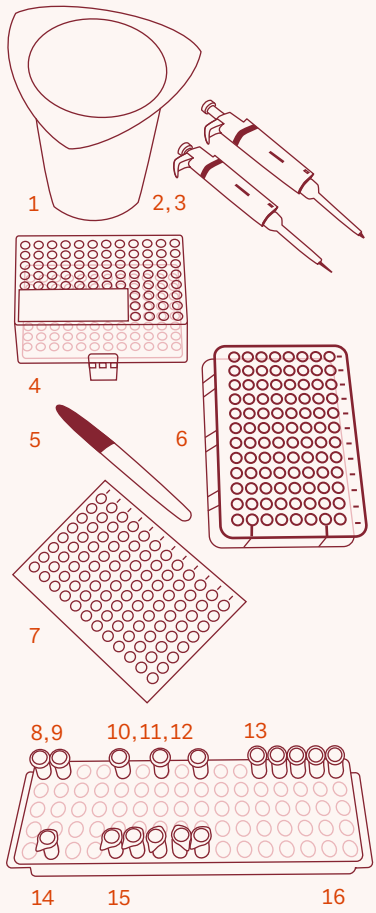
Preparation

2 | Anytime before the lab | 30 min

☐ Set up lab stations (1 per pair)

Note > *2 pairs of students will share reagents but should have their own set of all the other materials.*

1	Waste bucket
2	P20 micropipette
3	P200 micropipette
4	Micropipette tips
5	Permanent marker
6	96-well plate
7	96-well template card
8	Water
9	Antigen
10	Substrate
11	Blood plasma antibody
12	Labeled antibody
13	Participant samples (a, b, c, d, e, f)
14	1 empty 1.5-mL tube
15	5 empty 1.5-mL tubes for serial dilutions
16	Microtube rack



3 | After the lab | 15 min

☐ Properly dispose of lab supplies:

— *Any excess solutions can go down the drain*
— *Used micropipette tips and microtubes can go in the trash*

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Lab

Virtual Learning Options

1 Anytime — 30 min

☐ Ask students to explore an ELISA simulation like this one from HHMI Biointeractive (*ELISA Simulation*: read the captions below the pictures (left side) and the information in the lab notebook (right side) and complete the corresponding *capture sheet*)

2 After the ELISA simulation — 15 min

☐ Give students this completed data table and have them complete the rest of the Student Guide:

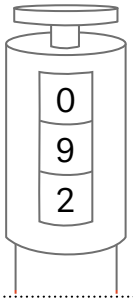
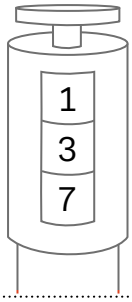
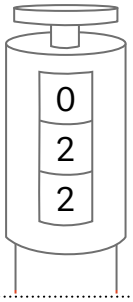
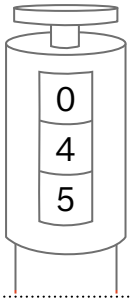
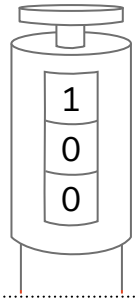
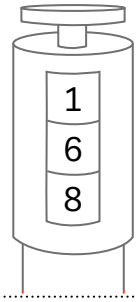
Sample	Color Signal	Sample	Color Signal
Tube #1	20.8	Participant a	11.1
Tube #2	9.8	Participant b	4.0
Tube #3	5.7	Participant c	17.6
Tube #4	2.3	Participant d	3.8
Tube #5	0.6	Participant e	12.4
		Participant f	18.5

Building Lab Skills: Micropipetting Practice

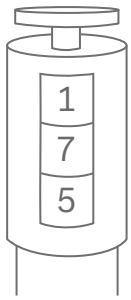
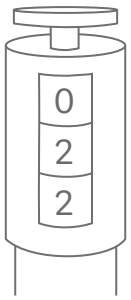
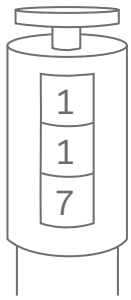
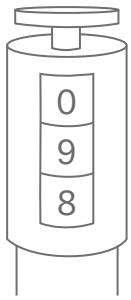
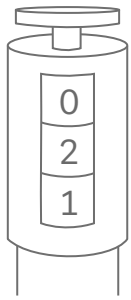
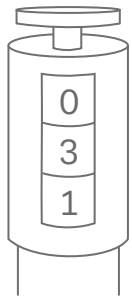
ANSWER KEY — **Do not share with students**

Directions
Complete the questions and activities below to practice using micropipettes.

1. Read the top and dial for each micropipette shown below, and write the volume.

P1000 200–1000 µL	P20 2–20 µL	P200 20–200 µL	P20 2–20 µL	P1000 200–1000 µL	P200 20–200 µL
					
920 µL	13.7 µL	22 µL	4.5 µL	1000 µL	168 µL

2. Choose the correct micropipette for each volume listed below and fill in the micropipette top and dial box.

P200	P1000	P20	P1000	P200	P20
					
175 µL	220 µL	11.7 µL	980 µL	21 µL	3.1 µL

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Building Lab Skills: Micropipetting Practice

ANSWER KEY **Do not share with students**

Continued

Micropipetting Practice: ROY GEE BIV

	Procedure	Which micropipette?
1	Add 38 µL of red water to tube #1.	P200 (038)
2	Add 44 µL of yellow water to tube #3.	P200 (044)
3	Add 50 µL of blue water to tube #5.	P200 (050)
4	Transfer 8 µL from tube #1 to tube #2.	P20 (080)
5	Transfer 8 µL from tube #1 to tube #6	P20 (080)
6	Transfer 8 µL from tube #3 to tube #4.	P20 (080)
7	Transfer 14 µL from tube #3 to tube #2.	P20 (140)
8	Transfer 14 µL from tube #5 to tube #4.	P20 (140)
9	Transfer 14 µL from tube #5 to tube #6.	P20 (140)

Reaction Tubes

All tubes should have 22 µL.

Analysis Questions

1. Describe ROY, Gee, and BIV's pattern:

Rainbow

2. Identify an error you made in accuracy and describe the evidence that this error occurred.

Answers will vary but should include rationale from *Make the Pattern and Check Your Accuracy* such as, the volumes in the tubes were not equal, there was air at the bottom of the tip (under-pipetting), or there was liquid left in the tube (over-pipetting).

3. How can you prevent this error in the future?

Answers will vary, but some possible answers include:
- Only depressing plunger to first stop (not second stop) when filling micropipette (results in over-pipetting)
- Releasing the plunger before taking the tip out of the receiving container (results in liquid not being ejected from tip)
- Turning the dial too far; all micropipettes should be used only within the range specified
- Using the incorrect micropipette resulting the wrong volume of liquid transferred

4. Write instructions describing how to use a micropipette. Use sequencing language to construct your response *(First,....Next,...After that,...Following that step... Finally,...).*

Answers will vary, sample response: First, you choose the correct micropipette for the volume you need to transfer and pick up a clean tip. Next, you push the plunger to the first stop. Then, you dip the tip into the liquid and slowly release the plunger to suck the liquid up. After that, you transfer the liquid into a tube or other vessel by pressing down to the first stop and then all the way to the second stop. You have to keep holding the plunger down while removing the tip from the tube or vessel. Finally, you can eject the tip into the trash by pressing the ejector button (some brands do not have this feature).

Serial Dilutions and the Standard Curve Questions

ANSWER KEY **Do not share with students**

Directions
Answer the questions below after working through the Building Lab Skills material.

1. When you performed a serial dilution, what did you notice about the color of each solution? How can this pattern be explained?

The more dilute the solution is, the lighter the color is. Because each solution is 10x less concentrated, the color should also be 10x lighter (but this is difficult to see with the naked eye).

2. If you were to make a standard curve of the serial dilution you performed, what would the next step be?

Measure the color signal of each solution using a spectrophotometer or smartphone app and then make a scatter plot graph of concentration vs. color signal.

3. Why might performing a serial dilution of a highly concentrated solution be a more accurate way to make a solution of very low concentration than making the lower concentration from scratch?

Making a solution of low concentration requires measuring out a very small mass or volume of the solute. This means any error from either the equipment or person doing the measuring will have a greater impact on the final concentration (any miscalibration or small mistake will greatly affect the concentration).

4. Why is a linear trendline the most appropriate type of equation for a standard curve (as opposed to exponential, polynomial, etc)?

There is a direct, 1:1 relationship between concentration and color signal from a solution.

5. Imagine you measured the color signal of an unknown solution and it was 8.71. Use the equation in the standard curve example to determine the concentration of this solution.

standard curve equation: $y = 20.5(x) + 0.103$

Concentration (mg/mL) = x

$8.71 = y$

$8.71 = 20.5\ (x) + 0.103$

x = 0.42 mg/mL

Vaccines and the Immune System Questions

ANSWER KEY **Do not share with students**

Directions
Answer the questions below after closely reading the background material.

1. Describe how a vaccine works as if you were explaining to a 1st grade science student. You may include a drawing to help you explain.

Answers may vary, but should demonstrate an understanding that the vaccine elicits an immune response. Example: A vaccine is a shot that teaches your body what a germ looks like so that if you ever catch that germ, your body will know how to fight it off.

2. What is one similarity and one difference between other modern vaccines and mRNA vaccines?

One similarity between other modern vaccines and mRNA vaccines is that they both stimulate your immune system to produce antibodies. Another answer could be that they both work to provide immunity to a particular disease.

One difference is that other modern vaccines contain a weakened or killed version of the virus or bacteria, whereas mRNA vaccines contain instructions for your cells to make a piece of the virus or bacteria.

3. Describe how the structure of an antibody helps it carry out its function?

Antibodies are Y-shaped proteins produced by immune cells that specifically recognize the proteins found on the surface of the virus. The antibody binds to the pathogen, immobilizing it and attracting cells that will engulf and destroy it.

4. How is the immune response different between the primary and secondary 'booster' vaccination? Use the graph to support your answer and include IgM and IgG antibodies in your explanation.

After the primary vaccination, IgM antibodies are produced first and the level increases over the first week or two. IgG antibody production begins about a week or two after vaccination, and these levels increase, while the IgM antibody concentration decreases. At the time of the booster vaccination, IgG antibodies still remain in the blood and are produced much faster and at much higher concentrations. A small amount of IgM antibodies are also produced.

5. What is a correlate of protection and why are researchers attempting to discover one for COVID-19 vaccines?

A correlate of protection is a measurable sign that a person is immune to a disease, typically a particular level of antibodies. Researchers are attempting to discover one for COVID-19 vaccines because it would give them a way to determine the efficacy of a vaccine without waiting to see if people contract the disease.

ELISA Questions

ANSWER KEY **Do not share with students**

Directions
Answer the questions below after closely reading the background material.

1. Describe one reason it is useful to be able to detect the amount of a certain protein in someone's blood.

If the protein is a biomarker of disease, detecting how much of it is in a person's blood could indicate whether or not they have the disease or their risk level of developing the disease.

2. If there was no color change (no signal) detected after performing an ELISA, what would this mean about the amount of antibodies to the virus in a person's blood? Explain step by step why this is.

No color change means that there were no (or very few) antibodies to the virus present in the person's blood plasma sample.

If no antibodies were present in the blood, nothing would bind the antigen that was captured on the surface of the wells. If no antibodies are bound to the antigen, the labeled antibody would have nothing to bind to. If no labeled antibodies are bound to blood plasma antibodies, the substrate has no enzyme to react with. If no enzymatic reaction occurs, no color change occurs.

3. If you forgot to add substrate during an ELISA, what would you expect to see in the 96-well plate. Use evidence from the steps to justify your prediction.

If you forgot to add substrate, you would expect to see no color change (no signal) even if there were antibodies to the virus present in the blood plasma. The substrate is necessary for the enzymatic reaction to happen with the enzyme attached to the labeled antibody. So if no substrate is added, no enzymatic reaction occurs, and therefore no color change occurs.

Student Guide, Part 1: Pre-Lab

ANSWER KEY — **Do not share with students**

1. Read through Steps #5–#10 in the Student Protocol, Part 1: Serial Dilution and ELISA. Draw a unique symbol for each of the ELISA components and the order in which they bind to each other in the 96-well plate.

1a. Symbol for labeled antibody

Can be anything — in reality the antibodies are Y-shaped

Example: 

1b. Symbol for a substrate

Can be anything—in reality the antibodies are Y-shaped

Example:

1c. Symbol for SARS-CoV-2 spike protein (antigen)

Can be anything — in reality the antibodies are Y-shaped

Example: 

1d. Symbol for IgG antibody (from blood sample)

Can be anything — in reality the antibodies are Y-shaped

Example: 

1e. Combine the symbols to show their binding order

2. Predictions:

2a. In which vaccine trial participants do you predict you will detect IgG antibodies to the spike protein on SARS-CoV-2? Explain your answer.

Examples:

All will have IgG antibodies because they received the vaccine and didn't get severe COVID-19

B and D will not have IgG antibodies because they developed mild COVID-19 symptoms

2b. Do you expect to see differences in IgG antibody concentration between any of the participant samples a–f? Explain your reasoning.

It may be expected to see lower concentrations of antibodies in participants b and d because they developed mild COVID-19 even after 2 doses of the vaccine. There also may be differences in how individual immune systems respond to vaccination that could lead to differences in IgG production.

2c. If there are no IgG antibodies in a sample, what would you expect to see in an ELISA well in terms of color signal (none, some, or high intensity)? Explain your answer with words or a drawing.

If there are no IgG antibodies, you would see no color signal because the substrate would have nothing to react with to produce a color chance. With no IgG, the labeled antibody that contains the enzyme required for the reaction has nothing to bind to.

Students could draw a well with no color/no light signal being produced.

2d. If there is a high concentration of IgG antibodies in a sample, what would you expect to see in an ELISA well in terms of color signal (none, some, or high intensity)? Explain your answer with words or a drawing.

If there is a high concentration of IgG antibodies, you would see a high intensity color signal. More IgG bound to the antigen leads to more labeled antibodies bound to IgG, which leads to more enzymatic reactions between the substrate and enzyme, which leads to a darker color.

Students could draw a well with a dark color/lots of light signal being produced.

Continues next page >

Student Guide, Part 1: Pre-Lab

ANSWER KEY **Do not share with students**

Continued

3. Serial Dilution: Read through Step #3 in the Student Protocol, Part 1: Serial Dilution and ELISA.

3a. What is in the stock solution you will be diluting and what is its concentration?

IgG antibodies to SARS-CoV-2 spike protein in a 1 µg/mL concentration.

3b. Why is this the appropriate solution for making the standard curve?

In order to determine the unknown concentration of these antibodies in participant blood samples, you need to have a set of known concentrations of the antibody to compare them to.

3c. Fill in the table below for each tube in the serial dilution. Tube #1 has been completed as an example.

	µL of water	µL of solution	Final IgG concentration (µg/mL)
1	0 µL water	200 µL of stock solution	1 µg/mL
2	100 µL water	100 µL of Tube #1	0.5 µg/mL
3	100 µL water	100 µL of Tube #2	0.25 µg/mL
4	100 µL water	100µL of Tube #3	0.125 µg/mL
5	100 µL water	—	0 µg/mL

4. Participant Samples: Read through Step #4 in the Student Protocol, Part 1: Serial Dilution and ELISA.

4a. What is in each "participant sample" and when was it collected?

Blood plasma from clinical trial volunteers collected 28 days after the second dose of an mRNA COVID-19 vaccine.

4b. What is "unknown" about each sample? (What are you measuring?)

The concentration of IgG antibodies to SARS-CoV-2 spike protein.

Student Guide, Part 2: Lab

ANSWER KEY — **Do not share with students**

Directions

Follow the Student Protocol, Part 1: Serial Dilution and ELISA to set up the lab with your partner.

1. Record 3 observations from your 96-well plate after completing the final incubation. Use your understanding of ELISA to explain what each observation means.

1a. Symbol and drawing for labeled antibody

Observations *I see...*	Explanations *This means...*
I see a color gradient from dark to light in wells C3–C7.	This means that as expected, each sample in the serial dilution has a lower concentration of IgG antibodies than the sample before.
I see the darkest well in row D is D5/D8.	This means that participant c/f had the highest concentration of IgG antibodies in their blood.
I see the lightest well is row D is D4/D6.	This means that participant b/d had the lowest concentration of IgG antibodies in their blood.

2. Follow the Student Protocol, Part 2: Color Signal and Standard Curve to collect the ELISA data with your partner and make a standard curve graph individually.

— After taking a screenshot of the color signal readings, record them in the data table below
— Attach an image of your standard curve graph

2a. Standard curve color signal and IgG Concentration from your data.

	Color Signal	IgG Concentration (µg/mL)
1	Answers will vary.	1 µg/mL
2	Answers will vary.	0.5 µg/mL
3	Answers will vary.	0.25 µg/mL
4	Answers will vary.	0.125 µg/mL
5	Answers will vary.	0 µg/mL

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Student Guide, Part 2: Lab

ANSWER KEY **Do not share with students**

Continued

2b. Participant color signal and IgG Concentration from your data and the class average.

Answers will vary.

	Color Signal	IgG Concentration (µg/mL) *Your Data*	IgG Concentration (µg/mL) *Class Average*
a			
b			
c			
d			
e			
f			

2c. Standard curve:

Answers will vary.

2d. Equation of standard curve $(y = mx + b)$:

Answers will vary.

2e. R^2 value:

Answers will vary.

Student Guide, Part 3: Data Analysis

ANSWER KEY — **Do not share with students**

Directions
Answer the questions below to analyze the results of your ELISA.

1. According to your R^2 value, is your standard curve reliable? How do you know?

The closer the R^2 value is to 1, the better the data fits the trendline, and therefore the more reliable your data is. If the R^2 value is less than 0.95, this indicates that the data points from your serial dilution do not form a straight line, meaning the standard curve will not reliably calculate the concentration of an unknown sample.

2. What does *y* represent in your standard curve equation? What does *x* represent?

y = color signal

x = IgG concentration (µg/mL)

3. Use your equation to calculate the concentration (in µg/mL) of IgG antibodies in each of the participant samples and complete the data table in Part 2. Show your work below:

Use each color signal from wells D3–D8 (participant samples) as *y* in the equation and solve for *x*.

Example:

Color signal for D8: 17.3

Equation: $y = 20.5(x) + 0.103$

$x = 0.84$ µg/mL (this is the concentration of the unknown sample)

4. Examine the class data for all of the IgG concentrations in the participant samples. Identify 3 patterns/observations in the data, using the following questions to guide you:

— What surprises or stands out to you?
— What patterns will help you answer the driving questions?
— How does the class data compare to your own data?

All participants should show the presence of IgG but the concentrations vary.

Participants c and f should have the highest IgG concentrations at around 0.9 µg/mL.

Participants b and d should have the lowest IgG concentrations at around 0.2 µg/mL.

Participants a and e should have medium IgG concentrations at around 0.6 µg/mL.

There will likely be outliers due to the high chances of micropipetting error in this experiment.

Continues next page >

Student Guide, Part 3: Data Analysis

ANSWER KEY **Do not share with students**

Continued

5. Describe two potential sources of error in this lab and how they may affect the results. Types of error include systematic and procedural, random, and human error:

   Possible answers might include:

	Source of error	How it may affect results
1	Inaccurate pipetting while making the serial dilution (ex. air bubbles in the tip while pipetting samples) (human)	An incorrect serial dilution will lead to a flawed standard curve graph which will result in the calculated antibody concentrations being incorrect. (Ex. air bubbles will lead to lower volumes)
2	Inaccurate pipetting/mixing up samples while transferring samples to the 96-well plate (human)	Standard curve graphs may be flawed or participant samples may be mis-read.
3	Contaminated tubes/wells (random)	Calculated antibody concentration would be artificially high.
4	Incorrect incubation time (human)	Not enough time for the enzymatic reaction to take place would result in not enough color signal to measure. Too much time could result in all the samples becoming too dark and obscuring differences between samples.
5	Small data set (only six participants) (Procedural/systematic)	These participants may not accurately represent the whole clinical trial—may show a skewed set of antibody concentrations.
6	No repeats of samples (only one well per participant) (Procedural/systematic)	Testing the samples in duplicate or triplicate would provide more statistically reliable data.
7	Testing the samples in duplicate or triplicate would provide more statistically reliable data.	Knowing the concentration of IgG antibodies before vaccination would provide stronger evidence for whether or not the vaccine triggered production of them.

Student Guide, Part 4: Arguing from Evidence

ANSWER KEY — **Do not share with students**

Directions

Follow the Student Protocol, Part 1: Serial Dilution and ELISA to set up the lab with your partner.

1. Did the vaccine trigger production of IgG antibodies to the SARS-CoV-2 spike protein? What evidence supports this?

Yes

- All participants have IgG concentrations above 0 µg/mL.
- None of the volunteers in the clinical trial tested positive for SARS-CoV-2 or reported COVID-19 symptoms before the trial and all received 2 doses of the vaccine, which provides evidence that the IgG antibodies were produced in response to the vaccine.

Cannot be determined

- Since no "before vaccination" blood samples were tested, there is not evidence for a triggering/increase in IgG production after vaccination.
- It is possible the participants had been asymptomatically infected with SARS-CoV-2 at some point before their negative test before the trial and this triggered their immune system to produce IgG antibodies.

2. Does the evidence you cited above support or refute your predictions in Part 1 a) and b)? Explain.

Answers will vary based on student predictions.

3. Compare the class data to the table in Part 1 about whether or not the participants contracted COVID-19. Can you conclude whether or not a particular concentration of antibodies confers immunity to COVID-19? What evidence supports this?

Participants b and d, who contracted mild COVID-19 after vaccination, have the lowest IgG concentration at around 0.2 µg/mL. The next lowest concentrations should be around 0.6 µg/mL. Therefore, it could be hypothesized that a concentration between 0.2 µg/mL– 0.6 µg/mL could be the correlate of protection (enough to confer immunity).

However, you cannot conclude that this this confers immunity because of the errors described in Part 3 #5.

4. If you were to design a follow-up experiment, what would you do to gather more evidence to determine the correlate of protection for COVID-19 vaccines? Explain why this would strengthen your findings.What surprises or stands out to you?

Ideas include:

- Correcting errors listed in Part 3 #5 by doing things like increasing the number of participants tested, measuring IgG concentration before vaccination as well as after, testing each sample in triplicate, etc.
- Testing blood samples from participants in a clinical trial for a different COVID-19 vaccine that was considered efficacious.
- Injecting a model organism (ex. Mice, rats, primates) with concentrations of IgG antibodies between 0.2 µg/mL–0.6 µg/mL, exposing them to SARS-CoV-2 and seeing if they contract COVID-19.

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Building Lab Skills: Precision Pipetting Cards

Directions
Use the appropriate micropipette to deliver the indicated volume of water in a single droplet on each space below.

P20				
2 µL	5 µL	10 µL	15 µL	20 µL

P200				
50 µL	75 µL	100 µL	150 µL	200 µL

P1000			
250 µL	500 µL	750 µL	1000 µL

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Building Lab Skills: Micropipetting Reference Guide

Key Vocabulary

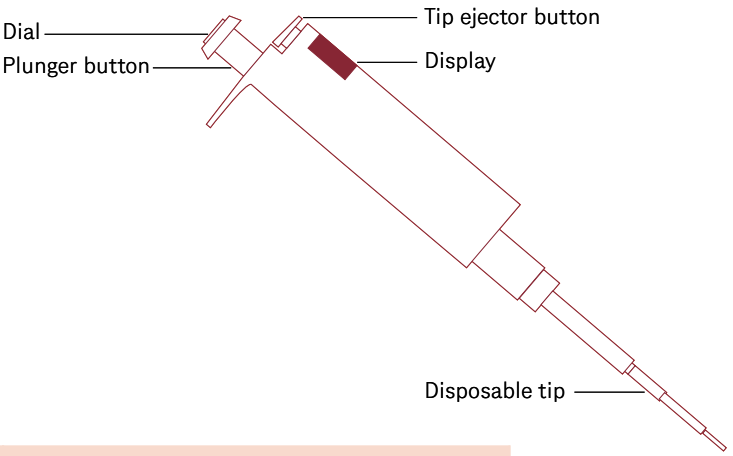
Micropipette

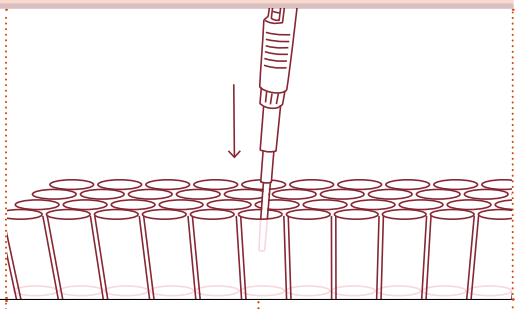
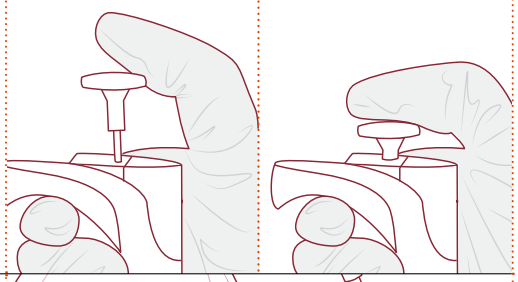
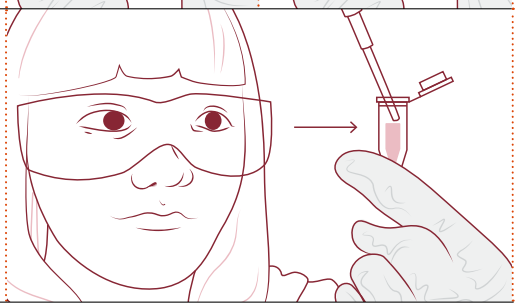
A laboratory tool commonly used in biology, biotechnology, and medicine to transport a very small volume of liquid. It is sometimes just called a pipette.

Microliter (µL):

A metric unit of volume that is measured using a micropipette. One microliter is one millionth of a liter.

Parts of a Micropipette



	Micropipetting Instructions	
1	**Place** a clean tip on the end. *Use firm, downward pressure.* *Use new disposable tip every time.* *The small tips (yellow or white) are for the P20 and P200.; the large tips (blue or white) are for the P1000.*	
2	**Press** the plunger down to the first stop and hold. *Use thumb.* *Keep vertical.*	
3	**Insert** the tip beneath the surface of the liquid. *Hold at eye level.*	

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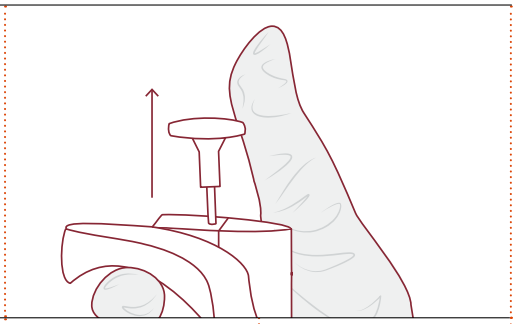
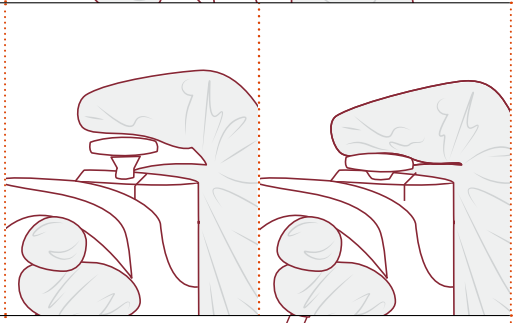
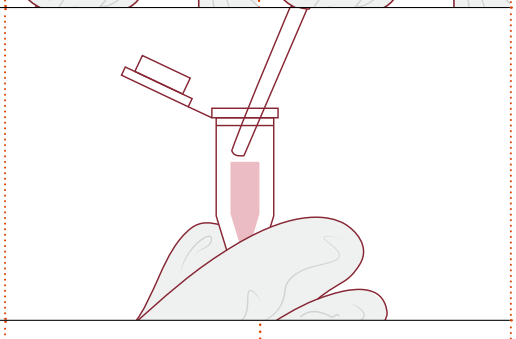
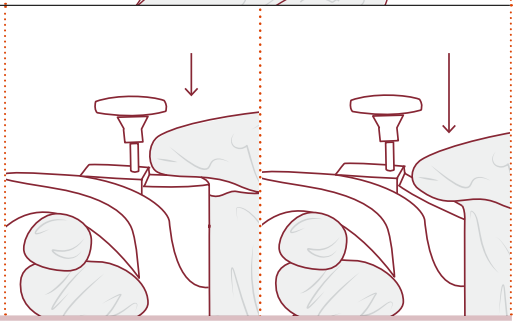
Building Lab Skills: Micropipetting Reference Guide

Continued

4	**Slowly release** the plunger to suck liquid into the tip. *Control the speed of the plunger with your thumb.* *Make sure the tip remains under the surface of the liquid as you release the plunger to avoid air bubbles.*	
5	**Dispense** liquid by pressing the plunger to the first stop, then the second stop, and then hold. *Touch the tip to the inside wall of the new tube .* *Go slowly.*	
6	**Remove** tip from sample. *Keep thumb down until the tip is out of tube.*	
7	**Discard** the tip by pressing the ejector button. *Never reuse a tip.* *Never touch a used tip.* *This is an example of sterile technique to prevent contamination between samples.*	

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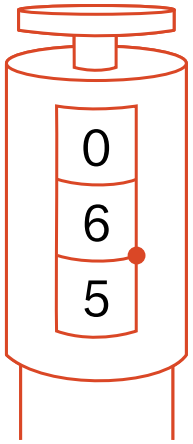
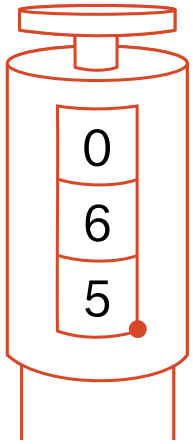
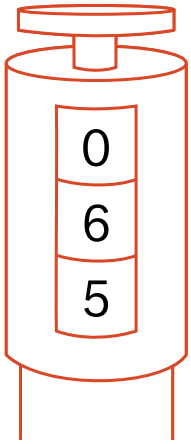
Building Lab Skills: Micropipetting Reference Guide

Continued

Choosing a Micropipette

● = decimal used to illustrate the concept, but does not show in actual display.

	P20 2–20 µL	P200 20–200 µL	P1000 200–1000 µL
Top			
Minimum Volume	2	20	200
Maximum Volume	20	200	1000
Dial			
	6.5 µL	65 µL	650 µL
	0 Tens (10) 6 Ones (1) 5 Tenths (0.1)	0 Hundreds (100) 6 Tens (10) 5 Ones (1)	0 Thousands (1000) 6 Hundreds (100) 5 Tens (10)

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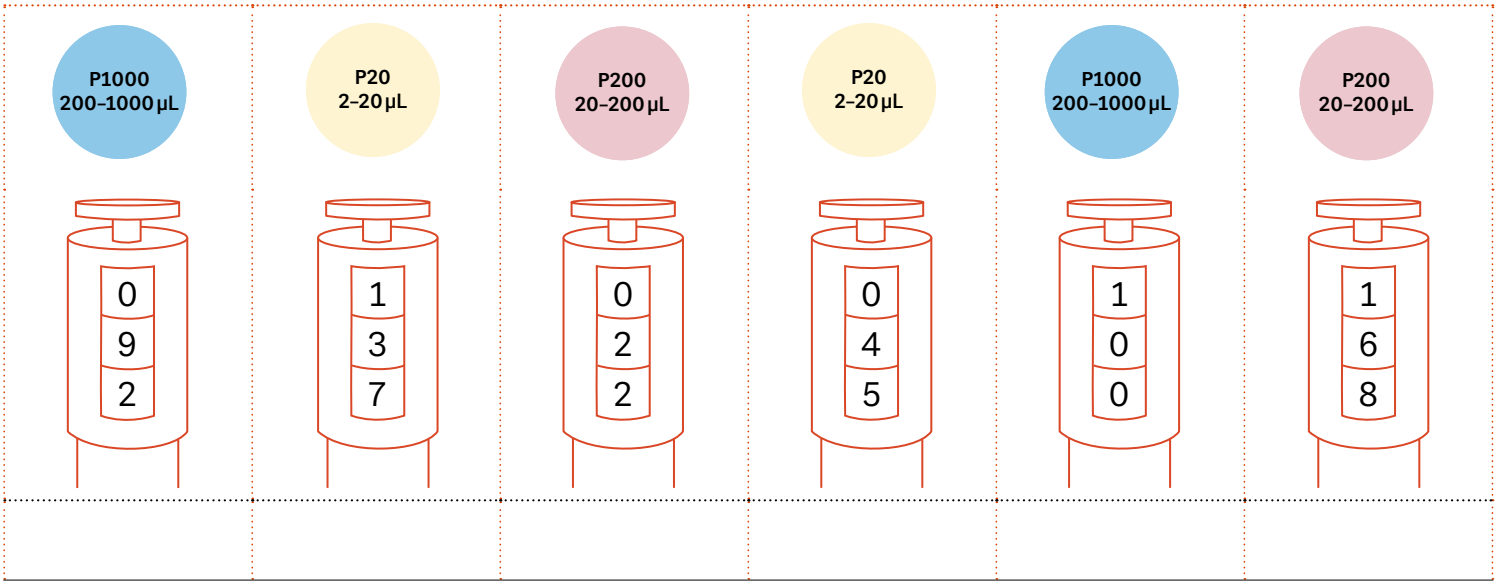
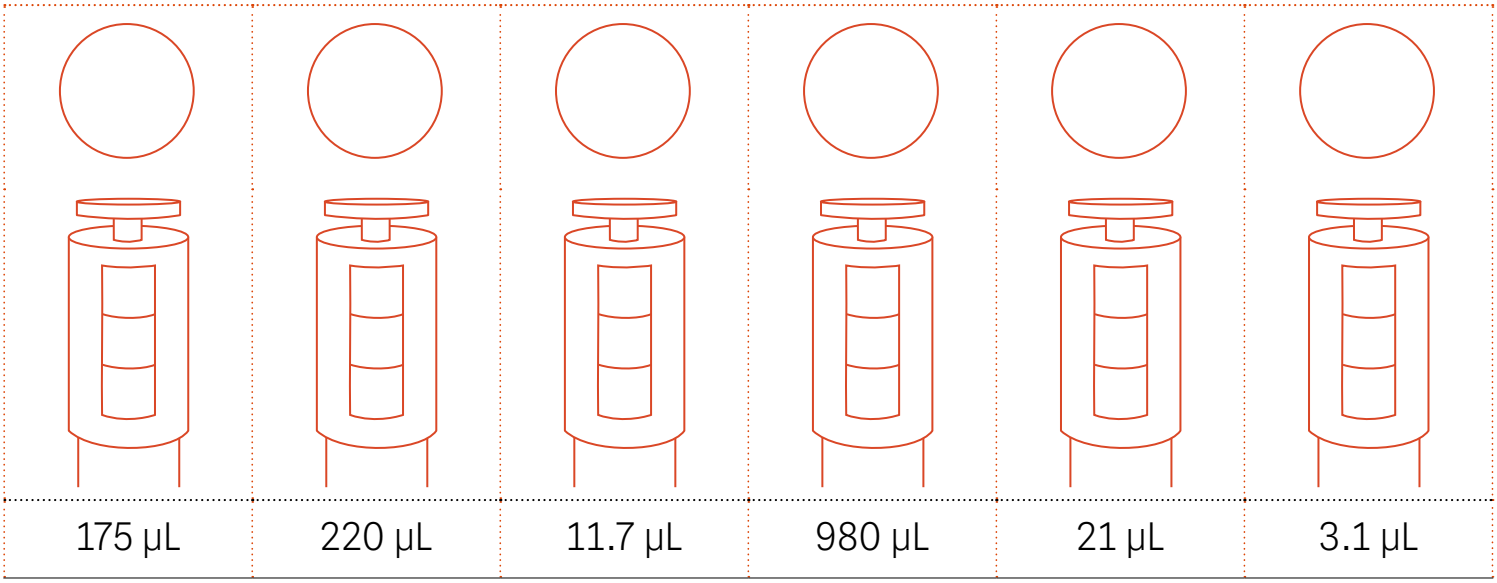
Building Lab Skills: Micropipetting Practice

Directions
Complete the questions and activities below to practice using micropipettes.

1. Read the top and dial for each micropipette shown below, and write the volume.



2. Choose the correct micropipette for each volume listed below and fill in the micropipette top and dial box.



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Building Lab Skills: Micropipetting Practice

Continued

3. Precision Pipetting Practice.

3a. Obtain practice cards for the micropipettes (with a waterproof surface) and a tube of colored liquid.

3b. Slowly micropipette each volume into the space below it on the card.

3c. Reminders:

— Set the dial before you put on a new tip.
— It is very important not to exceed the micropipette range when adjusting the dial as this can interfere with the calibration of the micropipette.
— Hold the micropipette at eye-level when withdrawing liquid form the tube, avoiding air bubbles.
— Release the liquid **slowly** and observe the size of the drop.
— Change the tip every time to ensure sterile technique.

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Building Lab Skills: Micropipetting Practice

ROY GEE BIV

Background

ROY, Gee, and BIV are supposed to micropipette liquid to make a pattern, but they don't remember what that pattern is supposed to be. Help them figure out the pattern by using the directions below and the most accurate pipetting technique possible.

Directions

1. *Complete the "Procedure" table below by deciding which micropipette (P20, P200, or P1000) you should use for each step and what the dial should look like for the listed volume.*
2. *Calculate the expected total volume for each tube and record it in the Reaction Tubes table.*
3. *Label each of six empty tubes #1–6 with permanent marker.*
4. *Complete each step of the procedure and check off each box as you go.*

	Procedure	Which micropipette?	Dial
1	Add 38 µL of red water to tube #1.		....
2	Add 44 µL of yellow water to tube #3.		....
3	Add 50 µL of blue water to tube #5.		....
4	Transfer 8 µL from tube #1 to tube #2.		....

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Building Lab Skills: Micropipetting Practice

ROY GEE BIV

Continued

	Procedure	Which micropipette?	Dial
5	Transfer 8 µL from tube #1 to tube #6.		
6	Transfer 8 µL from tube #3 to tube #4.		
7	Transfer 14 µL from tube #3 to tube #2.		
8	Transfer 14 µL from tube #5 to tube #4.		
9	Transfer 14 µL from tube #5 to tube #6.		

Continues next page >

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Building Lab Skills: Micropipetting Practice

ROY GEE BIV

Continued

Reaction Tubes

Tube #	Starting Volume	Calculated Total Ending Volume *Expected Result*	Accuracy + – √
1	38 µL		
2	0 µL		
3	44 µL		
4	0 µL		
5	50 µL		
6	0 µL		

Make the Pattern and Check Your Accuracy

1. Place your tubes in order and hold all six tubes up to eye-level. Do the volumes look the same?

   Yes No

2. Set your micropipette dial to the calculated expected volume for tube #1. Remove that volume from the tube and record your accuracy in the Reaction Tubes table by following the rules below:

– if there is air at the bottom of the tip (under-pipetting)

\+ if there is liquid left in the tube (over-pipetting)

√ if the tip is full, with nothing left in the tube. (great job!)

3. Eject each sample onto the paper towel in a line from left to right.

Continues next page >

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Building Lab Skills: Micropipetting Practice

ROY GEE BIV

Continued

Analysis Questions

1. Describe ROY, Gee, and BIV's pattern:

2. Identify an error you made in accuracy and describe the evidence that this error occurred.

3. How can you prevent this error in the future?

4. Write instructions describing how to use a micropipette. Use sequencing language to construct your response *(First,....Next,...After that,...Following that step... Finally,...).*

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Building Lab Skills: Serial Dilutions and the Standard Curve

Directions
Hands-on Practice: Perform a Serial Dilution.
Obtain the following materials and follow the steps below.

- P1000 micropipette and tips
- 4 test tubes (ex. Conical tubes) or small containers (10–50 mL)
- 1 graduated cylinder (10–50 mL)
- Labeling tape
- Permanent marker
- Stock solution

1. Label each tube A–D and add 9 mL of water to each using a graduated cylinder.

2. Use the P1000 to transfer 1000 µL (1 mL) of *stock solution to tube A*. This tube now contains 10 mL total (1 mL of stock solution + 9 mL of water). This means it is 1/10th the concentration of the stock solution. You just performed a 1/10 dilution.

3. Transfer 1 mL of *tube A solution to tube B*. This tube now contains 10 mL total (1 mL of A + 9 mL of water). This means it is 1/10th the concentration of A and 1/100th the concentrations of the stock solution. You just performed a 1/10 dilution.

4. Transfer 1 mL of *tube B solution to tube C.* This tube now contains 10 mL total (1 mL of B + 9 mL of water). This means it is 1/10th the concentration of B and 1/1,000th the concentrations of the stock solution. You just performed a 1/10 dilution.

5. Transfer 1 mL of *tube C solution to tube D*. This tube now contains 10 mL total (1 mL of C + 9 mL of water). This means it is 1/10th the concentration of C and 1/10,000th the concentrations of the stock solution. You just performed a 1/10 dilution.

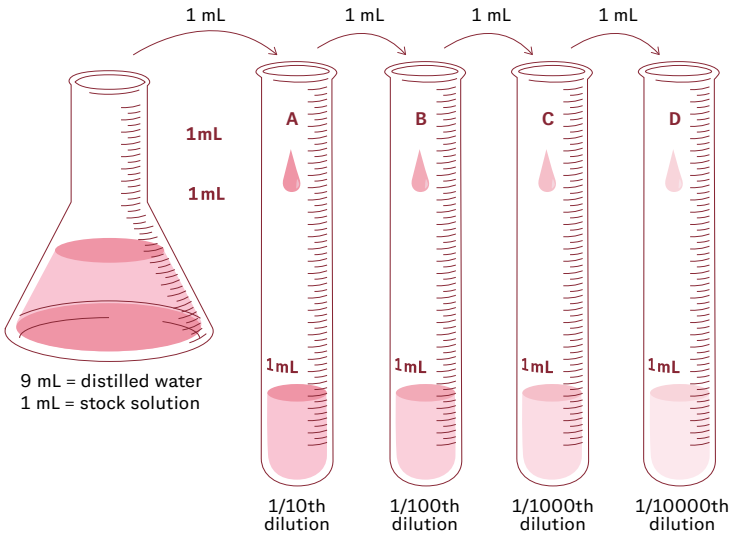
6. Answer the first question on your Serial Dilutions and the *Standard Curve Questions capture sheet*.

7. Clean up by pouring all solutions down the drain.

Reading: Serial Dilutions

When you *dilute* a solution, you add liquid so that it becomes less concentrated. For example, if your coffee is too strong, you could dilute it with water so that it is thinner and weaker. In a *serial dilution*, a stepwise sequence of dilutions is performed on a solution (think "serial" like "repeated"). It is a useful technique for making multiple solutions of very low concentrations quickly and accurately. The original solution is called the "standard solution" or *stock solution*, and is highly concentrated. In the image below, solution "D" is the result of a serial dilution on the original stock solution. It is 1/10,000th the concentration of the stock solution.

Serial Dilution Example



Each of the 4 dilutions in this example is a 1/10 ("one to ten") dilution. This is because each consists of 1 mL of the previous solution added to 9 mL of water, so 1 mL of solution in 10 mL total liquid.

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Building Lab Skills: Serial Dilutions and the Standard Curve

Continued

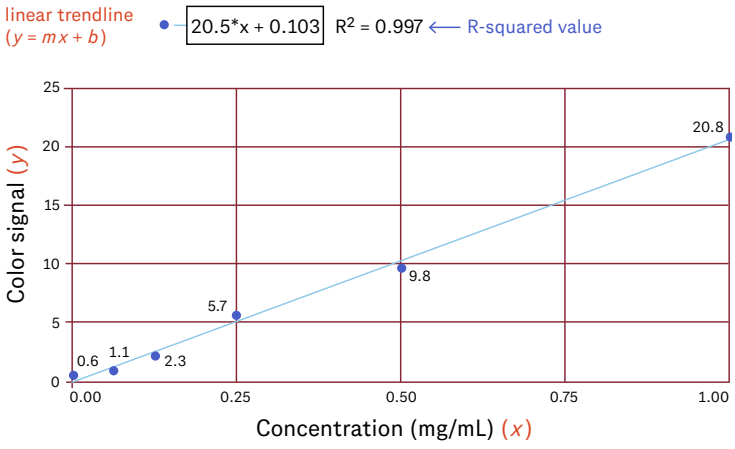
Standard Curve

Scientists often perform a serial dilution to make a standard curve (also called a calibration curve). A *standard curve* is a type of *graph* used to help the scientist determine the concentration of a compound in an unknown sample. It represents the relationship between the concentration of the compound you are interested in and the darkness of its color. In other words, it allows you to associate specific concentrations with specific *color signals* (the darker the color, the higher the concentration). Therefore, if you do not know the concentration of a sample, you can measure its color signal and then use the standard curve to calculate its concentration.

To make a standard curve graph:

1. Perform a serial dilution to make a set of *known concentrations.*
2. Measure the *color signal* for each concentration (using a spectrophotometer or smartphone app).
3. Graph a scatter plot with *concentration on the x-axis* and *color signal on the y-axis.*
4. Add a *linear trendline* to the scatter plot (this is the standard curve)!

Standard Curve Example



To use a standard curve graph:

Once you have the equation of your standard curve graph ($y = mx + b$), you can use it to calculate the concentration of an unknown sample. Measure the color signal for the unknown sample, substitute it for y and then solve for x, which represents the concentration.

Example using the adjacent standard curve:

Color signal for unknown = 17.3

$17.3 = 20.5(x) + 0.103$

$x = (17.3 - 0.103) / 20.5$

***x* = 0.84 mg/mL** (this is the concentration of the unknown sample)

You will also see a value called "R-squared" (R^2) for your linear trendline. This value measures how close the data fits the trendline. *The closer the R^2 value is to 1*, the better the data fits the trendline, and therefore the more reliable your data is. The R^2 of the example above is 0.997, which indicates a strong correlation between the data from the serial dilution and the trendline, meaning it can be reliably used to determine the concentration of an unknown.

If the R^2 value is less than 0.95, this indicates that the data points from your serial dilution do not form a straight line, meaning the standard curve will not reliably calculate the concentration of an unknown sample. Errors that could lead to this include:

- Inaccurate pipetting while making the serial dilution
- Contaminated tubes or wells
- Incorrect incubation time for producing a color signal (for an assay such as ELISA, the chemical reaction that results in the color signal takes a specific amount of time)

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Serial Dilutions and the Standard Curve Questions

Directions
Answer the questions below after working through the Building Lab Skills material.

1. When you performed a serial dilution, what did you notice about the color of each solution? How can this pattern be explained?

2. If you were to make a standard curve of the serial dilution you performed, what would the next step be?

3. Why might performing a serial dilution of a highly concentrated solution be a more accurate way to make a solution of very low concentration than making the lower concentration from scratch?

4. Why is a linear trendline the most appropriate type of equation for a standard curve (as opposed to exponential, polynomial, etc)?

5. Imagine you measured the color signal of an unknown solution and it was 8.71. Use the equation in the standard curve example to determine the concentration of this solution.

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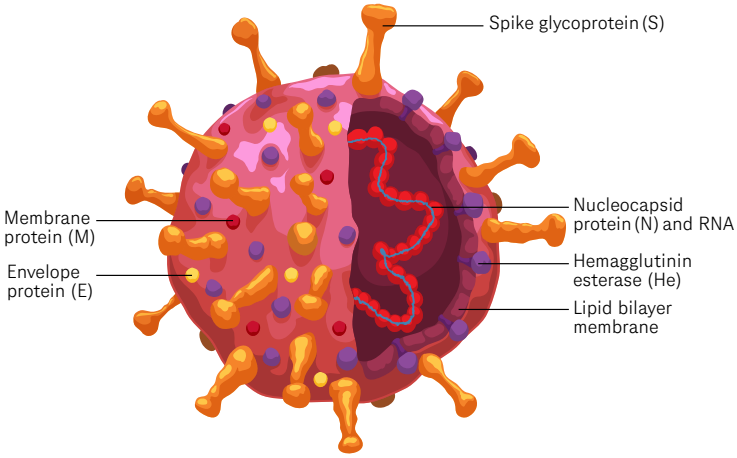
Background Reading: Vaccines and the Immune System

Modern Vaccines

Vaccines produced in the 19th and 20th century were made from viruses or bacteria that were attenuated (weakened) or killed. This strategy allowed the weakened pathogen to activate the immune system without causing disease. If the person became infected with the pathogen, the immune system was already trained to recognize it and carry out a quick and effective response. You can think of a vaccine like a training session for your immune system. In the late 1940s and beyond, scientists realized they could just use bits of the virus or bacteria to elicit the same immune response.

The image below shows some of the structures, such as viral proteins and nucleic acids, that can be used in a vaccine.

Virus Structure

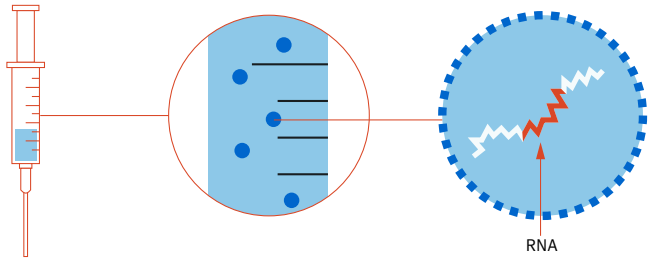


mRNA vaccines are a new type of vaccine that trains our cells to make a 'bit of virus', such as a protein that triggers an immune response inside our bodies. COVID-19 mRNA vaccines give instructions to our cells to make a harmless piece of protein called the "spike protein." The spike protein is found on the surface of the coronavirus SARS-CoV-2, the virus that causes COVID-19 (shown in orange in the image above). The immune system recognizes that the protein does not belong in the body and makes antibodies to fight what it thinks is an "infection" of coronavirus. At the end of the process, our bodies are trained how to protect against future infection.

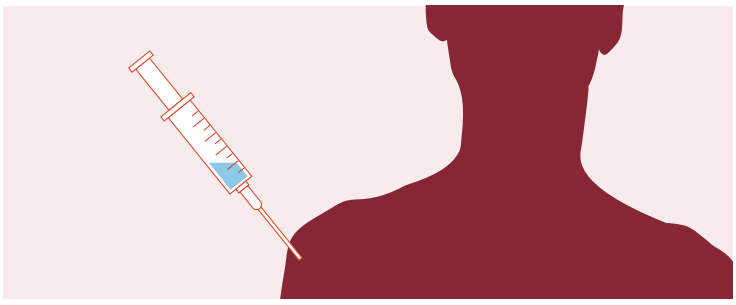
The image below shows how an RNA vaccine elicits the immune response.

How an RNA Vaccine Work

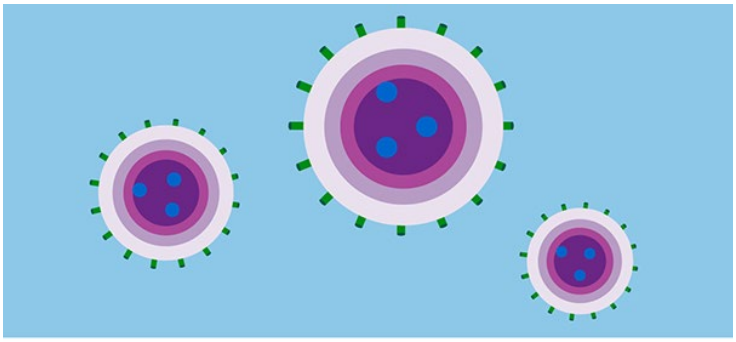
Scientists take part of the virus genetic code that tells cells what to build and coat it in a lipid so it can enter the body's cells.



This is injected into the patient.



The vaccine enters the cells and tells them to produce the coronavirus spike protein.



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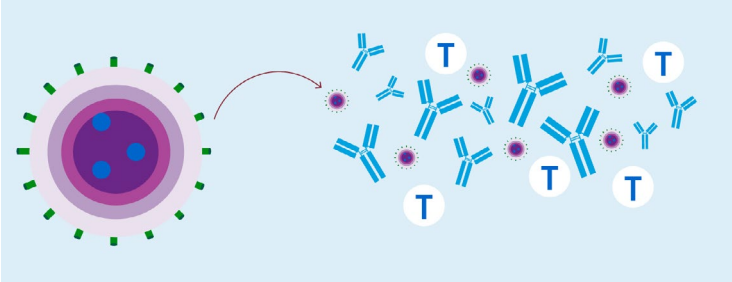
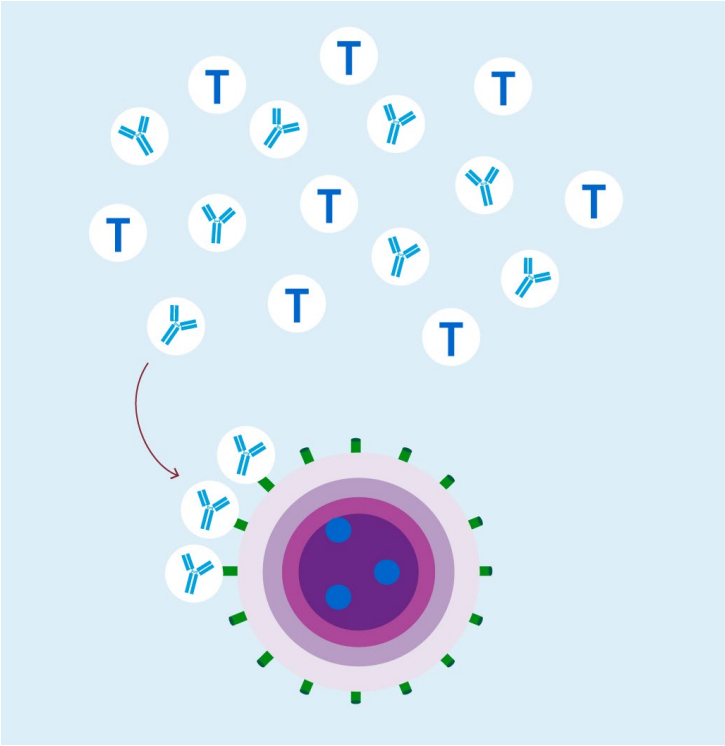
Background Reading: Vaccines and the Immune System

Continued

This prompts the immune system to produce antibodies and activate T-cells to destroy infected cells.



If the patient encounters coronavirus, the antibodies and T-cells are triggered to fight the virus.

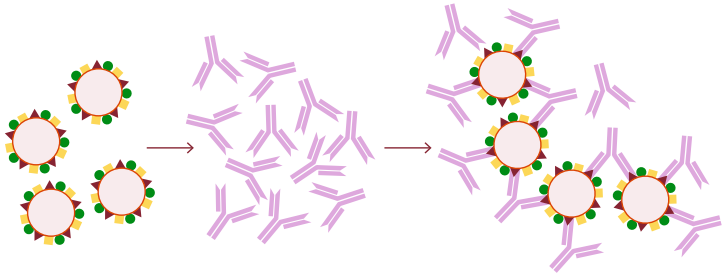


Antibodies and the Immune Response

Antibodies are Y-shaped proteins made as part of the body's immune response to an invading pathogen. When immune cells encounter a virus (or bacterium) for the first time, they produce antibodies that specifically recognize the proteins found on the surface of the virus. These antibodies are unique for each pathogen. For example, the antibody for chickenpox virus is not the same as the antibody for mononucleosis virus. The antibody binds to the pathogen, immobilizing it, and attracting other immune cells that will engulf and destroy it.

The image below shows how the unique structure of an antibody allows it to bind to the proteins found on the surface of the pathogens.

Antibodies selectively binding to a particular surface protein



Generally speaking, after recovering from an infection or receiving a vaccine, some of these antibody-producing immune cells remain in the body as memory cells. Because memory cells and antibodies are already present in your body, the next time you encounter the same pathogen, the immune response is much faster and more effective, stopping the infection from taking hold. This process is how we become immune to future infections of the same disease. However, like fingerprints, immune systems vary from person to person. Individual differences can be due to our history—who we live with and where we live—as well as genetics.

Continues next page >

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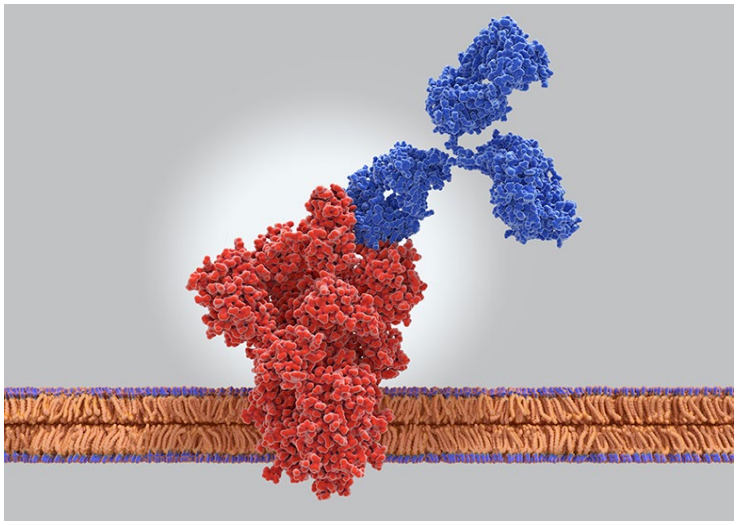
Background Reading: Vaccines and the Immune System

Continued

The image below shows an antibody binding to a spike protein of the coronavirus.

Antibody (blue) binding to a spike protein of the coronavirus (red)



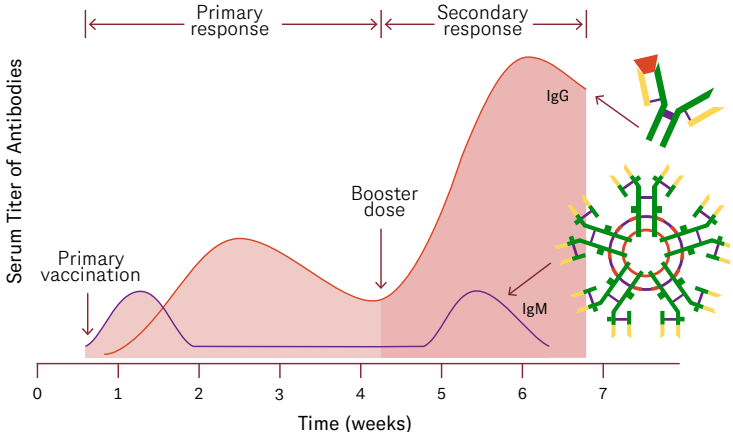
There are several types of antibodies, two important ones to know are:

- Immunoglobulin M (IgM) are the first antibodies produced by the immune system to fight a new infection, providing short-term protection. They increase for a few weeks and then decline as IgG production begins.
- Immunoglobulin G (IgG) antibodies are the most common (70-80% of the antibodies in the blood are IgG) and are produced 7 to 14 days after infection. They are detectable for months and even years, depending upon the pathogen and the individual, which helps protect you from a repeat infection.

Low IgM levels and high IgG levels indicate a past infection and possible immunity. Since SARS-CoV-2 is a new virus, we are still learning how our immune response works against COVID-19 and exactly how long antibodies last. New research is working to identify IgG levels that are required to ward off an infection.

The image below shows the concentration of IgM and IgG antibodies produced during the primary and secondary responses to a vaccine.

Concentration of IgM and IgG antibodies produced after vaccination



Vaccine Testing

Before a vaccine is broadly distributed to the general public, it must first go through a series of clinical trials to determine its safety and efficacy (*efficacy = the ability to produce a desired or intended result, in this case immunity to the disease*). An efficacy trial compares the rate of disease between vaccinated and unvaccinated people. For a vaccine to be considered efficacious, it means a very small proportion of vaccinated individuals contracted the disease compared with the number of unvaccinated people who contract the disease. A problem with this method of determining vaccine efficacy is that it is very time-intensive and resource-heavy. It requires thousands of volunteers and a lot of time and money spent monitoring individuals and waiting to see if they contract the disease.

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Background Reading: Vaccines and the Immune System

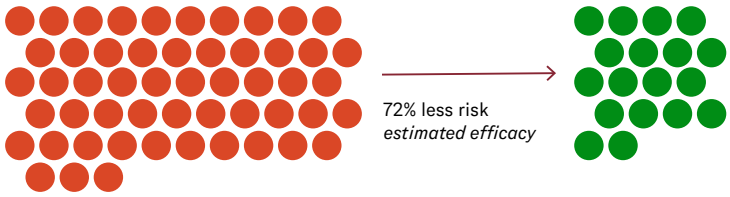
Continued

The image below demonstrates how vaccine efficacy is determined using the rate of disease compared between the vaccinated group and a placebo (unvaccinated) group.

Estimating vaccine efficacy

Placebo group
63 per 5,000 volunteers contracted COVID-19

Vaccinated group
18 per 5,000



A more efficient way to determine the efficacy of a new vaccine is to look for a biomarker in the volunteers' blood that indicates they have immunity to the disease. For example, if the vaccinated individuals' blood contains antibodies to the virus in a high enough concentration, you may be able to say the vaccine is efficacious. This measurement is called *correlate of protection*. The new influenza (flu) vaccine that is produced every year is tested using a correlate of protection. Researchers can check if the new flu vaccine causes individuals to make enough of a certain antibody, and if it does, it is considered efficacious.

Researchers have not yet discovered a correlate of protection for COVID-19 vaccines. In other words, we do not know whether the presence of certain antibodies indicate that someone is protected from contracting the disease. However, preliminary research in primates indicates that the presence of IgG antibodies to the spike protein in SARS-CoV-2, the virus that causes COVID-19, may protect against the disease. (In the image on the previous page, the spike protein is red and the IgG antibody is blue.) Once a correlate of protection is identified, clinical trials for new COVID-19 vaccines can speed up. This could pave the way for improved vaccines in the future, perhaps more versions that require only one dose or can be stored at warmer temperatures.

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References

Timeline | History of Vaccines

The History of Vaccines, From Smallpox to COVID-19 | Time

COVID-19 Vaccine Fact Vs. Fiction: An Expert Weighs in on Common Fears

Could a Blood Test Show if a Covid-19 Vaccine Works?

Understanding mRNA COVID-19 Vaccines

Antibody (Serology) Testing for COVID-19: Information for Patients and Consumers | FDA

Correlates of protection against SARS-CoV-2 in rhesus macaques

Immunological surrogate endpoints of COVID-2019 vaccines: the evidence we have versus the evidence we need

Efficacy and effectiveness | Immunisation Advisory Centre

Where you live shapes your immune system more than your genes

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Vaccines and the Immune System Questions

Directions
Answer the questions below after closely reading the background material.

1. Describe how a vaccine works as if you were explaining to a 1st grade science student. You may include a drawing between the dotted lines to help you explain.

2. What is one similarity and one difference between other modern vaccines and mRNA vaccines?

3. Describe how the structure of an antibody helps it carry out its function.

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Vaccines and the Immune System Questions

Continued

4. How is the immune response different between the primary and secondary 'booster' vaccination? Use the graph to support your answer and include IgM and IgG antibodies in your explanation.

5. What is a correlate of protection and why are researchers attempting to discover one for COVID-19 vaccines?

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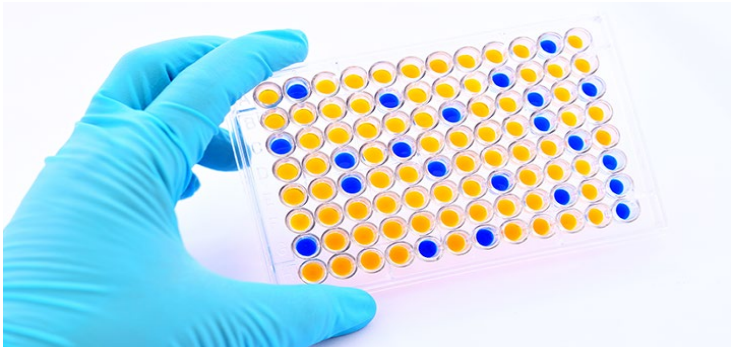
Background Reading: ELISA (Enzyme-linked Immunosorbent Assay)

Being able to detect the amount of a certain *protein* in a sample is an incredibly important tool in both scientific research and medicine. For example, many medicines are in fact proteins (such as insulin for people with diabetes), and many biomarkers of disease are also proteins (such as Prostate-specific antigen, which in certain levels may indicate risk of prostate cancer).

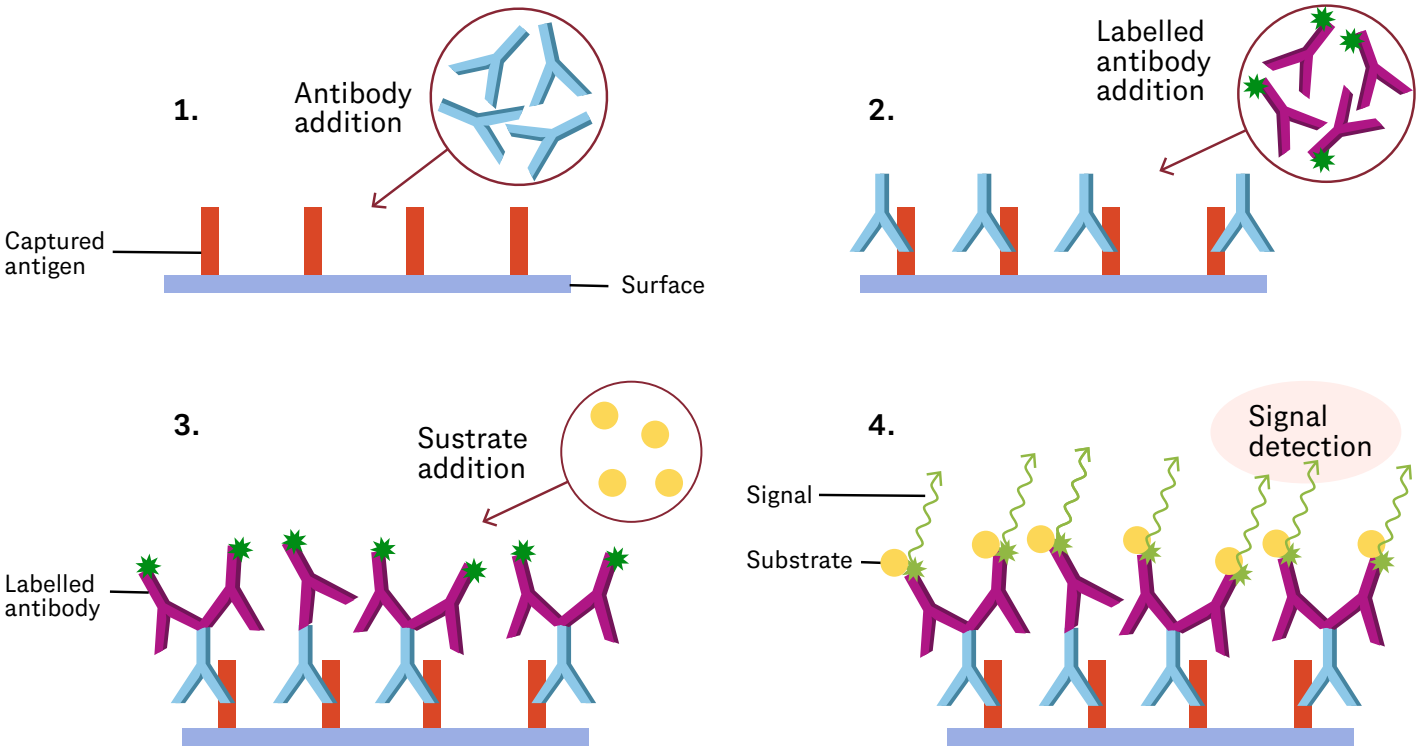
The antibodies that are produced by your immune system in response to infection with a virus or vaccination are also proteins. Therefore, in order to determine if a new vaccine "works," an important step is to detect the amount of antibodies made in an individual's blood after receiving the vaccine. One way scientists can do this by performing a type of test (also called an assay) called an *indirect ELISA*, which stands for "enzyme-linked immunosorbent assay."

This assay is conducted using a 96-well plate, a plastic dish containing 96 small wells in a 12x8 grid:

96-Well Plate



Main Components of an Indirect Elisa to Detect Antibodies in a Blood Plasma Sample



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Background Reading: ELISA (Enzyme-linked Immunosorbent Assay)

Continued

The table below summarizes the main components of an indirect ELISA to detect antibodies in a blood plasma sample:

Components	Description	Symbol
Antigen	First, a protein (or piece of a protein) is captured and immobilized on the bottom surface of each well in the 96-well plate. This protein will bind the antibodies you are trying to detect. For example, in an ELISA for COVID-19, the spike protein from the outside of the virus would be used to coat the bottom surface of the wells. (Step 1 in image)	
Blood Plasma Antibodies / Antibody addition	Next, blood plasma that may or may not contain the antibodies you are trying to detect are applied to the wells in the 96-well plate. The antibodies will recognize and bind to the antigen that was previously immobilized on the plate. (Step 2 in image)	
Labeled Antibody	An antibody (purple) that will recognize and bind to the blood plasma antibodies is then added to the wells. It has an enzyme attached to it as a "label" (green). (Step 2 in image)	
Substrate	Finally, a chemical that will bind to the enzyme, causing an enzymatic reaction to take place, is added. The reaction will cause a color change in the well and this signal can be measured. A darker color (stronger color signal) indicates there are more antibodies in the blood plasma sample. (Steps 3 and 4 in image)	

Text adapted from: *Four Types of ELISA-CUSABIO*

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ELISA Questions

Directions
Answer the questions below after closely reading the background material.

1. Describe one reason it is useful to be able to detect the amount of a certain protein in someone's blood.

2. If there were no color change (no signal) detected after performing an ELISA, what would this mean about the amount of antibodies to the virus in a person's blood? Explain step by step why this is.

3. If you forgot to add substrate during an ELISA, what would you expect to see in the 96-well plate. Use evidence from the steps to justify your prediction.

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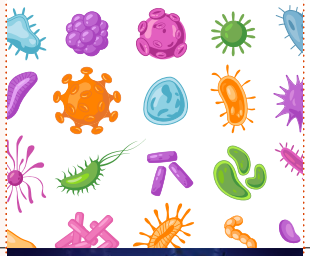
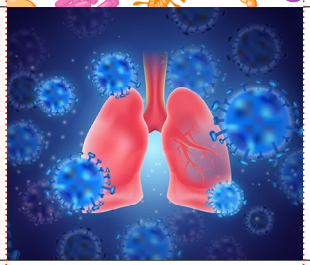
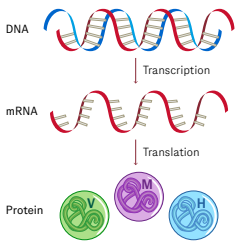
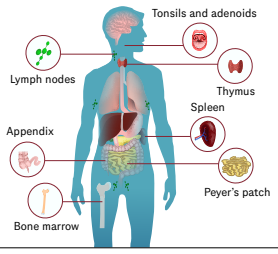
Vocabulary Tool

Directions
For each vocabulary word, write a new sentence that helps you practice using it.

Word	Image	Definition	Example Sentence	My Sentence
Vaccine		A substance used to stimulate the production of antibodies and provide immunity against one or several diseases.	An effective COVID-19 *vaccine* causes the person to make antibodies to the coronavirus and hopefully become immune to the disease.	
Pathogen		A microorganism that causes disease—for example, a bacterium or virus.	The coronavirus SARS-CoV-2 is the *pathogen* that causes COVID-19.	
COVID-19 *coronavirus disease 2019*		An infectious disease that primarily affects the respiratory system and is caused by the coronavirus SARS-CoV-2.	Some people who develop a severe form of *COVID-19* require hospitalization and ventilation, in which a machine assists their breathing.	
mRNA		A molecule that acts a messenger between the instructions held in DNA and the protein built from those instructions. It is made in a process called transcription.	Before a protein is made in the cell, DNA is transcribed into *mRNA*.	
Immune System		A system of cells, tissues, and organs that helps the body fight infections and other diseases.	One way your body's *immune system* responds to infection by a virus is to make antibodies to the virus.	

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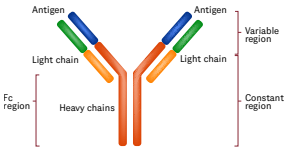
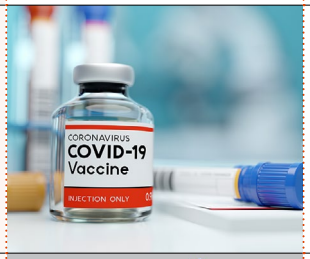
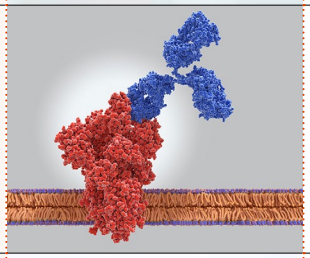
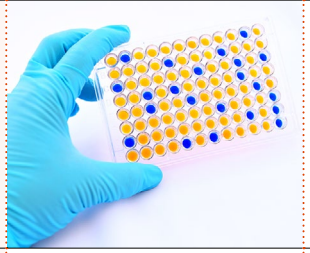
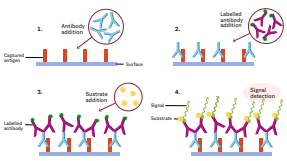
Vocabulary Tool

Continued

Word	Image	Definition	Example Sentence	My Sentence
Antibody		Y-shaped protein made as part of the body's immune response to an invading pathogen, such as a bacterium or virus.	An *antibody* to a virus will recognize and bind to a protein on the surface of that virus.	
Efficacy		The ability to produce an intended result, such as immunity to a disease.	In an *efficacy* trial, the rate of disease between vaccinated and unvaccinated volunteers is compared.	
Correlate of Protection		A biomarker that indicates immunity to a disease, often a certain amount of an antibody.	A certain concentration of IgG antibodies to SARS-CoV-2 spike protein is hypothesized to be a *correlate of protection* for COVID-19 vaccines.	
96-Well plate		A plastic dish containing 96 small wells in a 12x8 grid.	An ELISA is a type of test that is performed using *96-well plates*.	
ELISA (enzyme-linked immunosorbent assay)		A type of test used to determine the concentration of a protein (such as an antibody) in a sample.	One way to determine if someone who received a vaccine for a viral disease has antibodies in their blood to the virus is to perform an *ELISA*.	

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Student Protocol

Part 1: Serial Dilution and ELISA

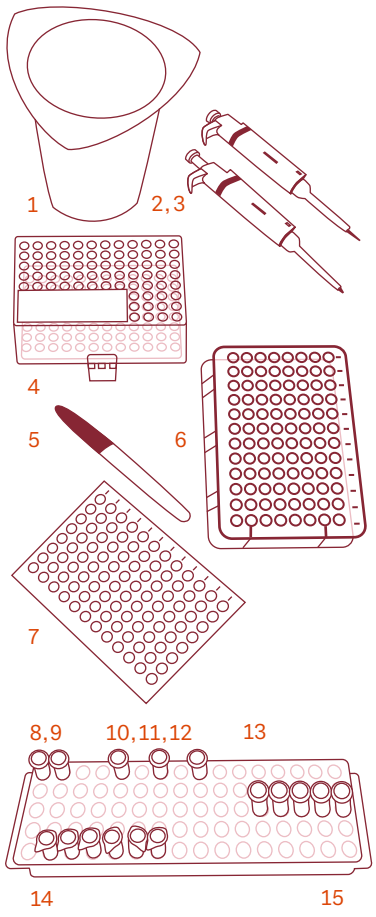
1 Preparations for the Lab

- ☐ Download a free microplate reader like *Spotxel Reader* app onto your smartphone. You can find it on Google play or iPhone app stores.
- ☐ Make sure your workspace is clean of clutter.
- ☐ Put on your gloves.

2 Clean the lab surface and gather all the materials needed to complete the lab:

☐

1	Waste bucket
2	P20 micropipette
3	P200 micropipette
4	Micropipette tips
5	Permanent marker
6	96-well plate
7	96-well template card
8	Water
9	Spike protein antigen
10	Substrate
11	Standard stock (1 µg/mL)
12	Labeled antibody
13	Participant samples a–f (blood plasma)
14	6 empty 1.5-mL tubes
15	Microtube rack



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Student Protocol

Part 1: Serial Dilution and ELISA

Continued

3	Serial Dilution for the Standard Curve (1 µg/mL)
☐	Label five 1.5-mL tubes 1–5 and place them in the microtube rack in order from 1–5.
☐	Pipette *100 µL water into tubes 2–5.*
☐	Pipette 200 µL of the *Standard Stock* into *tube 1*. This stock is a solution of IgG antibodies to the SARS-CoV-2 spike protein in a 1 µg/mL concentration.
☐	Pipette 100 µL of tube 1 contents into tube 2. Close the lid, flick gently to mix.
☐	Pipette 100 µL of tube 2 contents into tube 3. Close the lid, flick gently to mix.
☐	Pipette 100 µL of tube 3 contents into tube 4. Close the lid, flick gently to mix. Discard pipette.
☐	Leave tube 5 as is—it is the "0 µg/mL" control.
4	Determine the placement of the 11 wells you will use on the 96-well plate.
☐	Obtain a 96-well plate.
☐	Starting at well C3 of the template card, label the well #1, then on C4 label #2 etc. directly on the circles such that when you place the plate over the template, you can see the numbers. You will use these wells for your serial dilution (#1, #2, #3, #4, #5)
☐	Starting at D3, label the wells on the card with a, b, c, d, e, f for the participant samples. These are blood plasma samples taken 28 days after the second dose of the vaccine.
☐	Place the plate over the card so that the numbers are visible when you look down at the wells.

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Student Protocol

Part 1: Serial Dilution and ELISA

Continued

5	Pipette 100 µL Spike Antigen into all the labeled wells.
☐	This is the spike protein that coats the outside of SARS-CoV-2 and it will bind to the plate.

Note > *For the next set of steps, it is critical that you pay close attention to the reagent and the wells. Decide who will be the pipetter and who will be the quality checker. The quality checker will handle the reagents to make sure the pipetter puts them in the correct well. Switch roles for step 7.*

6	Pipetting serial dilution
☐	Pipette 50 µL of Standard tube #1 into the labeled well #1.
☐	Pipette 50 µL of Standard tube #2 into the labeled well #2.
☐	Repeat until all 5 tubes are in their correct wells.
7	Pipetting participant samples
☐	Pipette 50 µL of Participant “a” into the labeled well “a”.
☐	Pipette 50 µL Participant “b” into the labeled well “b”.
☐	Repeat with Participants “c–f” into the labeled wells “c–f”
☐	Mix gently by tapping the edge of the plate with the palm of your hand.
8	Incubate at room temperature for 10 minutes.

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Student Protocol

Part 1: Serial Dilution and ELISA

Continued

9	While you are waiting, dilute the substrate because it needs to be made fresh before use. This is a 1:1 dilution.
☐	Obtain a clean, clear 1.5-mL microtube and label it with “Dil” on top
☐	Pipette 75 µL of water into the “Dil” microtube.
☐	Add 75 µL of the substrate tube to the “Dil” tube.
☐	Close the tube, flick gently to mix. Fling to make sure the liquid is at the bottom of the tube or spin in a microfuge if available.
10	Add 10 µL of labeled antibody to each well.
11	Add 10 µL of the diluted substrate “Dil” to each of the 11 wells. Tap gently to mix.
12	Incubate at room temperature for at least 30 minutes and up to 24h.
☐	At this time, you should start to see a purplish color develop. Wait for the whole 30 minutes before continuing.
☐	If saving the plate to read another day, seal with the plate sealer or parafilm to avoid evaporation.

Potential Stopping Point—you can wait 30 minutes or take a picture the next day.

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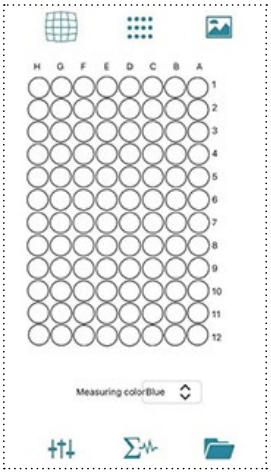
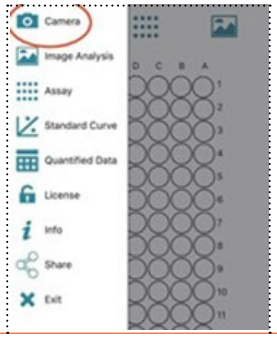
Student Protocol

Part 2: Color Signal and Standard Curve

1	Measuring Color Signal
☐ A	Go to *Apple* or *Android* Play Store and download a microplate reader such as *Spotxel® Reader* (free) if you have not already done so.
☐ B	Video instructions of using Spotxel *here*.
☐ C	Place your 96-well plate on a clean, white surface in bright light. The backside of the template card makes a good surface.
☐ D	Open the Spotxel app to use the in-app camera. It should look like this. 
☐ E	*Swipe right* and choose “Camera” from the menu or just swipe right to get to camera view (Android). You should see an outline of a 96-well plate over the camera image. 

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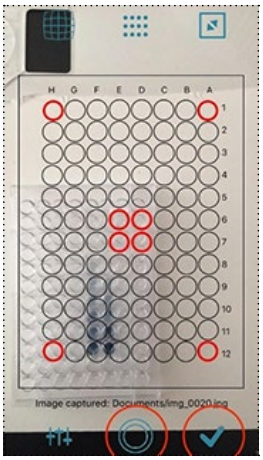
Student Protocol

Part 2: Color Signal and Standard Curve

Continued

☐ F Position the outline over the actual plate. Use the corner wells outlined in red to help you overlay the plate outline over the plate.



☐ G Click the circles icon at the bottom to take a picture. Be patient, this may take a while. If you like the picture, click the check mark to continue.

☐ H Move the plate outline again to align especially to the wells you used in the experiment. You can also stretch the circles if needed.

☐ I Once your outline is aligned, click the ∑ analysis button (bottom center). Wait for it to analyze the image, which may take a few minutes.

Note > *This picture was taken without the white background.*

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Student Protocol

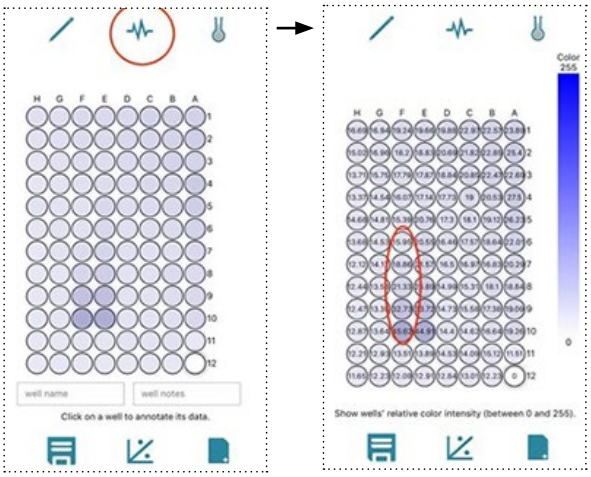
Part 2: Color Signal and Standard Curve

Continued

☐ J The jagged red circles represent the edges of the wells detected by the app. If they seem relatively well aligned with the microwell plate, *swipe left*.

Note > *This picture was taken without the white background.*

☐ K The darkness of the wells corresponds to the color intensity signal detected by the app. Click the squiggly analysis button (top center) to display the color signals.



☐ L Screenshot the color signal readings. The readings from the serial dilution samples will be used to make a standard curve graph. The readings from the participant samples will be used to determine the concentration of antibodies in the blood using the standard curve.

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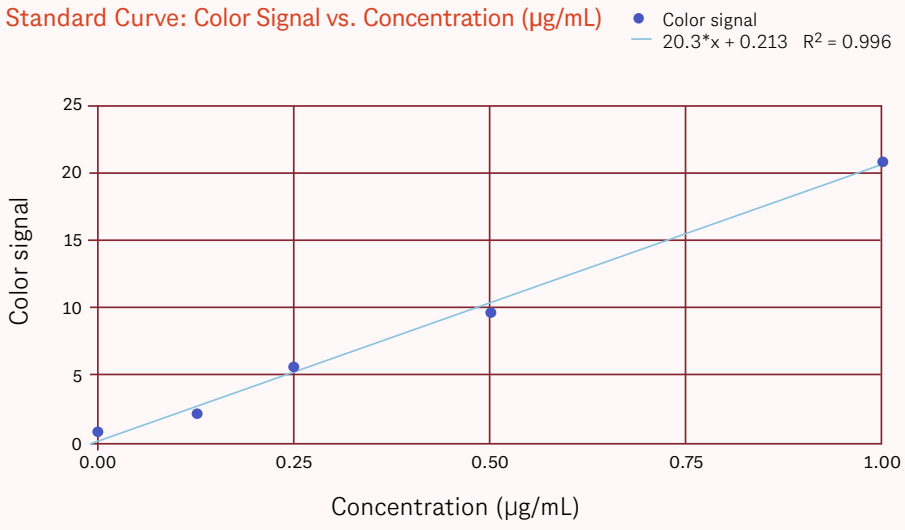
Student Protocol

Part 2: Color Signal and Standard Curve

Continued

2	Graphing a Standard Curve
☐ A	Open a new spreadsheet in a program such as Google Sheets or Microsoft Excel.
☐ B	In cell A1 type “Concentration (µg/mL)” and in B1 type “Color Signal.”
☐ C	In A2–A6 enter the concentration of each serial dilution sample (tubes #1–#5).
☐ D	In B2–B6 enter the corresponding color signal for each serial dilution sample (wells #1–5).
☐ E	Make a scatter plot of the data table. If using Google Sheets, highlight the entire table (A1–B6) and click Insert → Chart. It should automatically insert a scatter plot.
☐ F	Add a linear trendline to the scatter plot. If using Google Sheets, click Edit Chart → Customize → Series and check the box that says “Trendline.”
☐ G	Display the trendline’s equation on the graph. If using Google Sheets, click Edit Chart → Customize → Series and select “Use Equation” from the “Label” drop-down menu.
☐ H	Display the trendline’s R^2 value on the graph. If using Google Sheets, click Edit Chart → Customize → Series and check the box that says “Show R^2”.
☐ I	Standard Curve: Color Signal vs. Concentration (µg/mL)



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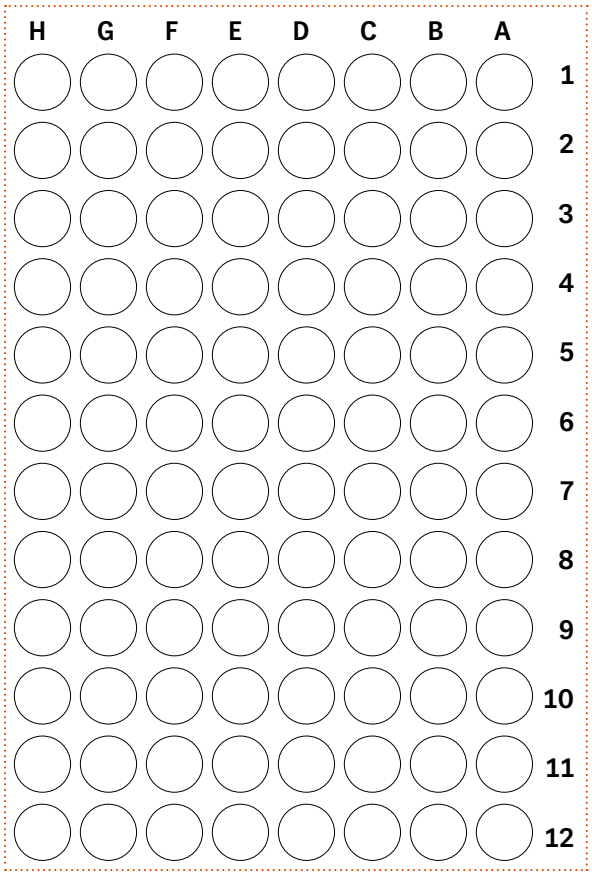
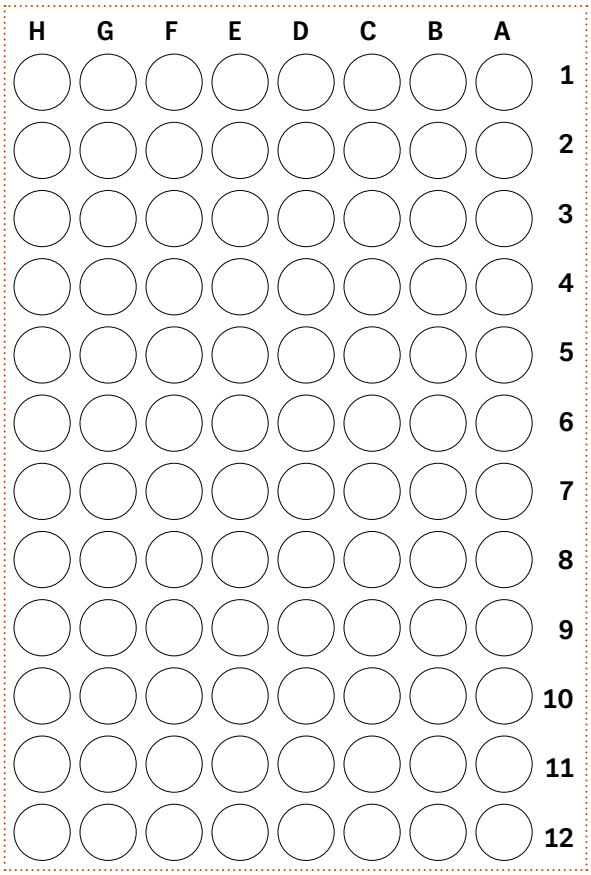
96-well Plate Template Card

Directions

Cut out the template cards and provide one to each pair of students to use as the set up their ELISA.





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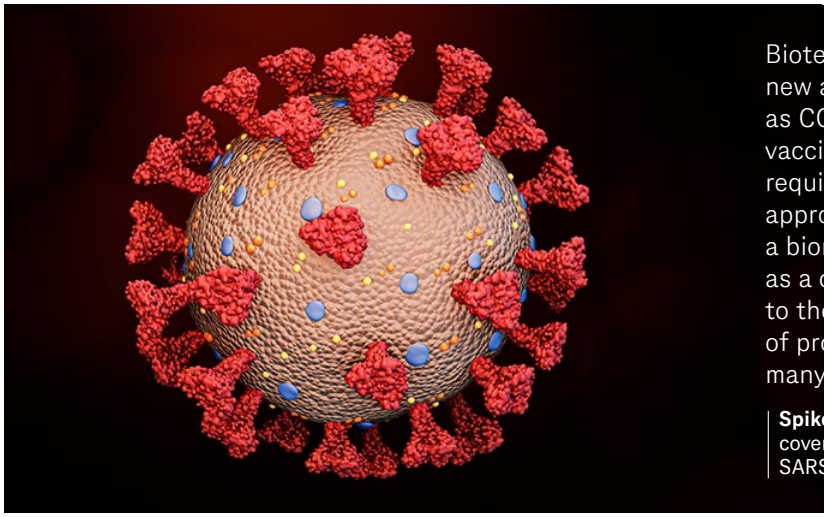
Student Guide, Part 1: Pre-Lab

In this lab, you play the part of a Vaccine Researcher working to discover a correlate of protection for COVID-19 vaccines. A previous experiment conducted in primates provided evidence that IgG antibodies to the spike protein on SARS-CoV-2 (the virus that causes COVID-19) provides protection against the disease. Your goal is to build on this research to confirm whether these antibodies are a biomarker for immunity to COVID-19 and if so, what concentration is required for immunity.

Driving Questions

How do we know the vaccine triggered production of IgG antibodies to the SARS-CoV-2 spike protein?

From this data, can you conclude whether or not a particular concentration of antibodies confers immunity to COVID-19?



Biotech companies around the world constantly develop new and improved vaccines for virus-caused diseases such as COVID-19. However, one of the barriers for making new vaccines is the time and resource-heavy clinical trial process required to demonstrate their efficacy. A more streamlined approach to showing that a new vaccine works is to look for a biomarker in the blood of vaccinated individuals, such as a certain amount of an antibody, that indicates immunity to the disease. This measurement is called the "correlate of protection" and it has not yet been discovered for many vaccines.

Spike proteins in red covering the surface of SARS-CoV-2

Directions

Analyze blood samples from six clinical trial volunteers who received a widely distributed mRNA vaccine.

This vaccine showed 93% efficacy during its clinical trials, meaning only a tiny portion of those receiving the vaccine developed mild COVID-19. To compare IgG concentrations between vaccinated individuals who were protected against COVID-19 (the vast majority) and those who contracted a mild form of the disease even after vaccination (the very small minority), analyze blood samples for the following six volunteers. Blood samples were taken 28 days after the second vaccine dose—generally the peak of antibody production. No volunteers in the clinical *trial tested positive for SARS-CoV-2 or reported COVID-19 symptoms before the trial.*

Vaccinated Clinical Trial Participants*

Participants	a	b	c	d	e	f
Did they contract COVID-19?	No	Yes Mild	No	Yes Mild	No	No

*
This is only a tiny subset of the thousands of participants in the clinical trial.

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FUTURELAB+

Student Guide, Part 1: Pre-Lab

Continued

1. Read through Steps #5–#10 in the Student Protocol, Part 1: Serial Dilution and ELISA. Draw a unique symbol for each of the ELISA components and the order in which they bind to each other in the 96-well plate.

1a. Symbol for labeled antibody

1b. Symbol for a substrate

1c. Symbol for SARS-CoV-2 spike protein (antigen)

1d. Symbol for IgG antibody (from blood sample)

1e. Combine the symbols to show their binding order

Continues on next page >

FUTURELAB+

Student Guide, Part 1: Pre-Lab

Continued

2. Predictions

2a. In which vaccine trial participants do you predict you will detect IgG antibodies to the spike protein on SARS-CoV-2? Explain your answer.

2b. Do you expect to see differences in IgG antibody concentration between any of the participant samples a–f? Explain your reasoning.

2c. If there are no IgG antibodies in a sample, what would you expect to see in an ELISA well in terms of color signal (none, some, or high intensity)? Explain your answer with words or a drawing.

2d. If there is a high concentration of IgG antibodies in a sample, what would you expect to see in an ELISA well in terms of color signal (none, some, or high intensity)? Explain your answer with words or a drawing.

Continues on next page >

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Student Guide, Part 1: Pre-Lab

Continued

3. Serial Dilution: Read through Step #3 in the *Student Protocol, Part 1: Serial Dilution and ELISA*.

3a. What is in the stock solution you will be diluting and what is its concentration?

3b. Why is this the appropriate solution for making the standard curve?

3c. Fill in the table below for each tube in the serial dilution. Tube #1 has been completed as an example.

Tube	µL of water	µL of solution (note tube #)	Final IgG concentration (µg/mL)
1	0 µL water	200 µL of stock solution	1 µg/mL
2			
3			
4			
5			

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Student Guide, Part 1: Pre-Lab

Continued

4. Participant Samples: Read through Step #4 in the *Student Protocol, Part 1: Serial Dilution and ELISA.*

4a. What is in each "participant sample" and when was it collected?

4b. What is "unknown" about each sample? (What are you measuring?)

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Student Guide, Part 2: Lab

Directions
Follow the Student Protocol, Part 1: Serial Dilution and ELISA to set up the lab with your partner.

1. Record 3 observations from your 96-well plate after completing the final incubation. Use your understanding of ELISA to explain what each observation means.

Observations *I see...*	Explanations *This means...*

2. Follow the Student Protocol, Part 2: Color Signal and Standard Curve to collect the ELISA data with your partner and make a standard curve graph individually.

— After taking a screenshot of the color signal readings, record them in the data table below.
— Attach an image of your standard curve graph.

2a. Standard curve color signal and IgG Concentration from your data.

	Color Signal	IgG Concentration (µg/mL)
1		1 µg/mL
2		0.5 µg/mL
3		0.25 µg/mL
4		0.125 µg/mL
5		0 µg/mL

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Student Guide, Part 2: Lab

Continued

2b. Participant color signal and IgG Concentration from your data and the class average.

	Color Signal	IgG Concentration (µg/mL) *Your Data*	IgG Concentration (µg/mL) *Class Average*
a			
b			
c			
d			
e			
f			

2c. Standard curve:

2d. Equation of standard curve $(y = mx + b)$:

2e. R^2 value:

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Student Guide, Part 3: Data Analysis

Directions
Answer the questions below to analyze the results of your ELISA.

1. According to your R^2 value, is your standard curve reliable? How do you know?

2. What does *y* represent in your standard curve equation? What does *x* represent?

3. Use your equation to calculate the concentration (in µg/mL) of IgG antibodies in each of the participant samples and complete the data table in Part 2. Show your work below:

4. Examine the class data for all of the IgG concentrations in the participant samples. Identify 3 patterns or observations in the data, using the following questions to guide you:
   - What surprises or stands out to you?
   - What patterns will help you answer the driving questions?
   - How does the class data compare to your own data?

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Student Guide, Part 3: Data Analysis

Continued

5. Describe two potential sources of error in this lab and how they may affect the results. Types of error include systematic or procedural, random, and human error:

	Source of error	How it may affect results
1		
2		

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Student Guide, Part 4: Arguing from Evidence

Directions

Answer the questions below to help you construct an argument that answers the driving questions.

1. Did the vaccine trigger production of IgG antibodies to the SARS-CoV-2 spike protein? What evidence supports this?

2. Does the evidence you cited above support or refute your predictions in Part 1 a) and b)? Explain.

3. Compare the class data to the table in Part 1 about whether or not the participants contracted COVID-19. Can you conclude whether or not a particular concentration of antibodies confers immunity to COVID-19? What evidence supports this?

4. If you were to design a follow-up experiment, what would you do to gather more evidence to determine the correlate of protection for COVID-19 vaccines? Explain why this would strengthen your findings. What surprises or stands out to you?

Continues on next page >

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Student Guide, Part 4: Arguing from Evidence

Continued

5. Use the organizer below to write a complete Claim, Evidence, Reasoning paragraph that answers the Driving Question: Did the vaccine trigger production of IgG antibodies to the SARS-CoV-2 spike protein and can you conclude if a particular concentration of IgG antibodies confers immunity to COVID-19?

CER Organizer

Optional Sentence Frames/Guiding Questions	
Claim	Statement that answers the driving question
Evidence: data collected from the lab	What were the spike protein IgG concentrations of the participants after vaccination (include units)?
	Is there other relevant information about the participants in the vaccine trial?
	Given sources of error and limitations of the investigations, how reliable are your results?
Reasoning: What scientific principles explain the data?	The evidence supports/does not support _____ because _____ .
	We can/cannot determine _____ because _____.
	_____ indicates that _____ because _____ .
	_____ explains why _____ .
	_____ occurs as a result of _____ .

Key Vocabulary
COVID-19
IgG antibodies
Concentration
SARS-CoV-2 spike protein
Vaccine trial
Correlate of protection
ELISA (enzyme-linked immunosorbent assay)

CER Paragraph:

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CER Rubric

Score	4	3	2	1
Claim	In the first sentence, a claim is made that clearly and specifically answers the guiding question.	In the first sentence, a claim is made that clearly answers the guiding question.	A claim is made that answers the guiding question. This claim is unclear OR not in the first sentence.	A claim is made but does not answer the guiding question.
Evidence	At least 3 pieces of evidence are provided that strongly support the claim. The evidence is very clear (including data analysis and comparison), logical, and relevant to the claim.	At least 3 pieces of evidence are provided that support the claim. The evidence is mostly clear, logical, and relevant to the claim.	Some evidence (at least 2 pieces) is provided that supports the claim.	Very little evidence (1 piece) is provided that supports the claim. Evidence is irrelevant, unclear, or illogical.
Reasoning	The reasoning uses the evidence to communicate the claim in a convincing way with significant use of scientific principles. Language is clear, explicit, and thorough.	The reasoning clearly and accurately relates the evidence to the claim with some use of scientific principles or real-world connections.	The reasoning begins to relate the evidence to the claim. There is some relevant reasoning, but not enough.	The reasoning attempts to relate the evidence and the claim.
Final Score				