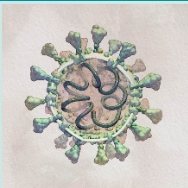


Biology of SARS-CoV-2

Click & Learn
Educator Materials

OVERVIEW

SARS-CoV-2 is a virus that, starting in 2020, has caused the largest global pandemic in recent history. The disease caused by this virus, called COVID-19, has affected millions of people worldwide. *Biology of SARS-CoV-2* is a four-part animation series that explores the biology of the virus, including the structure of coronaviruses like SARS-CoV-2, how they infect humans and replicate inside cells, how the viruses evolve, methods used to detect active and past SARS-CoV-2 infections, and how different types of vaccinations for SARS-CoV-2 prevent disease.

The accompanying "Student Worksheets" incorporate concepts and information from the animations. The "Version 1" worksheet was written by a high school educator and is appropriate for general high school biology students. The "Version 2" worksheet was written by a college educator and is appropriate for AP/IB Biology and undergraduate students. The worksheets can be edited or combined based on your learning goals.

This document contains multiple resources for using the animations with students, including the following (click links to go directly to each section):

- specific pause points for the animations with content summaries and questions
- general discussion points for the animations
- answer keys for the "Version 1" and "Version 2" worksheets
- references that provide more background on the science in the animations and worksheets

Additional information related to pedagogy and implementation can be found on this resource's webpage, including suggested audience, estimated time, and curriculum connections.

KEY CONCEPTS

- Viruses with an RNA genome have their RNA translated into proteins by an infected cell.
- Viruses hijack cellular machinery to make viral proteins, replicate, and spread to other cells.
- Mutations are random and can have positive, negative, or no effects on viruses.
- Mutations in the viral genome can be used to track how a virus is spreading through populations.
- Diagnostic tests for viral infections can detect viral RNA, viral antigens, or antibodies the body has produced in response to the virus.
- Vaccines protect from future disease by delivering antigens that trigger an immune response without causing an actual infection.
- Vaccines can deliver a weakened or inactivated form of the virus, antigen proteins, or genetic instructions such as DNA or mRNA.

STUDENT LEARNING TARGETS

- Identify structural components of SARS-CoV-2.
- Describe the steps in the SARS-CoV-2 replication cycle.
- Explain how mutations arise in the viral genome.
- Describe how a virus can change over time due to mutations.
- Outline several different ways to detect a viral infection.
- Describe how different types of vaccines expose the immune system to specific antigens.
- Explain how antigens stimulate a natural immune response, including the concepts of antibodies and immune memory.

PRIOR KNOWLEDGE

Before watching the **animations**, students should have a basic understanding of:

- what genetic mutations are
- the flow of genetic information from DNA to RNA to proteins
- the immune system

Before completing the **"Version 1" worksheet**, students should have a basic understanding of:

- the definition of a genome as the genetic makeup of an organism or virus
- the function of the viral envelope
- how vaccines work

Before completing the **"Version 2" worksheet**, students should have a basic understanding of:

- the ways in which cell types differ, including in their cell surface proteins
- the process of DNA replication
- terms associated with DNA and RNA replication, such as template, polymerase, and elongation
- analyzing and interpreting DNA sequence data, including how differences in DNA sequences can be used to track evolution

PAUSE POINTS

The animations may be viewed in their entirety or paused at specific points to review content with students. The table below lists suggested pause points, indicating the beginning and end times in minutes for each of the four animations.

For *Infection*:

	Begin	End	Content Description	Review Questions
1	0:00	0:39	• Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a member of the coronavirus family that causes a disease called COVID-19.	• When a person gets a nasal swab to see if they have been infected by SARS-CoV-2, it is typically called a "COVID-19 test." What would be a more accurate name for this test?
2	0:40	0:50	• Coronaviruses can be found in many animals, including humans. • Coronavirus diseases in humans range from mild to severe.	• What types of animals carry coronaviruses? • Does SARS-CoV-2 cause the common cold?
3	0:51	1:13	• The structure of a coronavirus includes an RNA genome, a membrane called the envelope, and spike proteins (called "protein spikes" in the animation).	• What type of genome do coronaviruses have? How does that differ from the human genome? • Why is this family of viruses called coronaviruses?
4	1:14	1:35	• After entering the human body, a virus gets into a cell and releases its genome inside the cell.	• How does SARS-CoV-2 enter the body? • Once inside the body, how does the virus gain entry into a cell?
5	1:36	2:08	• The virus genome is translated, replicated, and packaged inside the human cell.	• Why do you think the virus is able to use the human cell's ribosomes but not the cell's polymerase?
6	2:09	2:35	• The virus spreads to new cells and individuals, prompting an immune response and symptoms.	• Can a person with no symptoms spread SARS-CoV-2?

For *Evolution*:

	Begin	End	Content Description	Review Questions
1	0:00	0:43	• The SARS-CoV-2 genome is a single-stranded RNA of about 30,000 nucleotides that encodes fewer than 30 proteins.	• Does the length of the virus's genome or the number of proteins it encodes surprise you? Why or why not?
2	0:44	1:24	• SARS-CoV-2 replicates its genome by producing complementary template RNAs, which are used to make new RNAs that match the virus's genome. • Mutations occur randomly during the genome replication process. • Mutations occur when nucleotides are added, left out, or substituted for a different nucleotide.	• What are three ways in which nucleotides may be changed, resulting in a mutation?
3	1:25	2:21	• Mutations that provide a selective *advantage* help a virus spread through a population. • Mutations that provide a selective *disadvantage* hinder a virus's ability to spread. • Neutral mutations have no effect on a virus's ability to spread.	• Would a mutation that becomes *more* common in a population over time be most likely to provide a selective advantage, provide a selective disadvantage, or have no effect? • Why might a mutation that has a positive effect on the virus have a negative effect on humans?
4	2:22	2:47	• Tracking mutations in viruses is helpful for understanding viral spread and developing treatments for viral diseases.	• Why is tracking virus evolution so important?

For *Detection*:

	Begin	End	Content Description	Review Questions
1	0:00	1:03	• Available tests for SARS-CoV-2 assess whether a patient has an active infection or a past infection. • One test for an active infection is the RT-PCR test, which detects viral RNA.	• Which part of the virus does the RT-PCR test detect? • Is the RT-PCR test effective while the patient is currently infected or after they have recovered?
2	1:04	1:21	• Another test for an active infection is the antigen test, which detects viral proteins.	• Which part of the virus does the antigen test detect? • What are antigens?
3	1:22	1:49	• One test for a past infection is the antibody test, which detects whether the immune system has produced antibodies to fight off the virus.	• What does the antibody test detect? • What are antibodies?
4	1:50	2:22	• Each of the three types of tests has strengths and weaknesses and can be used to find out different information about an infection.	• Based on the graph at 2:03, when might it be more appropriate to get an antibody test than an RT-PCR or antigen test? • What is a false negative?

For *Vaccination*:

	Begin	End	Content Description	Review Questions

1	0:00	0:56	• The immune response is how the immune system responds to a viral infection. It includes the production of many cell types that help destroy the virus. • One cell type, B cells, produces antibodies that bind to protein antigens on the surface of the virus. • Antibodies help prevent the virus from entering cells and target it for destruction.	• What is the immune response? • What are antigens and antibodies? Which comes from the virus, and which is made by the body? • How do antibodies help protect us from viruses?
2	0:57	1:25	• The immune response converts some B cells into memory B cells, which can stay in the body for years and protect the body from infection. • Without memory B cells, it takes more time to produce enough antibodies to fight a virus. In the meantime, the virus may cause serious disease.	• When someone says their body "remembers" a particular virus, what does that mean? How is that memory acquired?
3	1:26	3:29	• Vaccines deliver antigens to trigger an immune response without causing disease. • Vaccines for SARS-CoV-2 deliver antigens in three main forms: inactive whole virus, antigen proteins, and genetic instructions (mRNA or DNA).	• How does a vaccine help prevent disease? • Why don't vaccines cause the same symptoms as the virus? • Why do you think vaccine developers might select one vaccine type over another?
4	3:30	4:05	• While the vaccines themselves are eliminated from the body, the memory B cells produced by the immune response to the antigens can last for years, providing protection. • Additional doses, called booster shots, may be needed to keep antibody levels high. • New vaccines may be needed as the virus mutates.	• Some SARS-CoV-2 vaccines require two doses. Why?
5	4:06	4:30	• Vaccinated people limit the virus's ability to spread within a population.	• Herd immunity describes the point at which a population has enough vaccinated people to protect the full population. Do you think every person within the population needs to be vaccinated to achieve herd immunity? Why or why not?

DISCUSSION POINTS

For *Infection*:

- After students watch this animation, assess their understanding of how a coronavirus replicates. You may want to introduce the terms "positive-sense" and "negative-sense" when talking about RNA, then ask students to explain the replication of SARS-CoV-2 using these terms.
  - If necessary, explain that the genome of SARS-CoV-2 is a positive-sense, single-stranded RNA, which means that it can be used as mRNA in the cell. From that "mRNA," the cell's ribosomes

translate viral RNA polymerase, which is necessary to replicate the viral genome. The polymerase transcribes the virus's positive-sense genome into a negative-sense template strand, which is used to make more copies of the genome. This replication cycle is illustrated within the coronavirus section of the *Virus Explorer* Click & Learn.

- Consider having students do the *Virus Explorer* Click & Learn to learn more about the structure and replication of viruses. The Click & Learn includes a description of "sense" and has a detailed diagram of the coronavirus replication cycle that students can explore and discuss.

For *Evolution*:

- Have students consider news headlines alerting that SARS-CoV-2 is mutating. Ask whether mutations in the virus will always be bad for humans or not.
  - One point that you may want to emphasize is that most mutations are neutral, and mutations that provide a strong selective advantage or disadvantage are relatively rare. This is not explicitly mentioned in the animation.

For *Detection*:

- You may want to extend students' understanding of the RT-PCR test by discussing how it works.
  - In particular, the virus's RNA genome must be transcribed into DNA before it can be sequenced. First, the enzyme reverse transcriptase (from retroviruses) is used to transcribe the RNA into DNA. Then, the polymerase chain reaction (PCR) is used to make many copies of the DNA for sequencing.
- Consider discussing pros, cons, and students' questions about the three tests (RT-PCR test, antigen test, and antibody test) in the animation.
  - The FDA's Coronavirus Testing Basics page provides more information on these tests, including how samples are taken, how long it takes to get results, and potential caveats.
  - SARS-CoV-2 antibody tests in particular currently have many open questions. (How long does it take antibodies for SARS-CoV-2 to be produced? How long do these antibodies last after infection? Do these antibodies protect a person from future infections? How many people would need antibodies for the population to be protected?)
    - You could ask students to consider why these tests might still be useful to perform, even if we don't know the answers to all the questions above. Students could also discuss potential negative consequences to performing these tests without more information.
- Consider connecting the concepts in this animation to SARS-CoV-2 testing recommendations that students may have heard about.
  - For example, when a person comes in close contact with someone who has tested positive for SARS-CoV-2, the Centers for Disease Control (CDC) have recommended that the person quarantine for 14 days, regardless of whether they have tested negative on the RT-PCR test or if they have any symptoms. Ask students to discuss the reasoning behind this recommendation. (Can a person be infected without symptoms? How might a person test negative and still be infected?)

For *Vaccination*:

- You may want to clarify that the immune response and vaccines can protect the body from many pathogens, not just viruses. For example, there are also vaccines for diseases caused by bacteria, such as tetanus, diphtheria, and pertussis (whooping cough).
- You may want to clarify that B cells and antibodies are only part of the immune response. Other immune cells, such as T cells, play major roles as well. Like B cells, T cells can also become memory cells that help protect the body from future infections.
- Students may know the vaccines by their trade names rather than by their mechanisms. Here are examples of each vaccine type and companies that are developing them:

- Inactive whole virus: Sinovac, Sinopharm
- Protein antigen: Novavax
- DNA: CanSino, AstraZeneca, and Johnson & Johnson
- mRNA: Pfizer, Moderna

- For the "Version 2" worksheet, consider relating student answers to Question 20 to the three tests (RT-PCR test, antigen test, and antibody test) discussed in the *Detection* video. Then discuss how these tests can avoid false positives from certain vaccines.
  - The RT-PCR test does not detect the genetic instructions (DNA or mRNA) vaccines because it detects a different region of the viral genome than the region that encodes the spike protein (which is delivered by these vaccines).
    - To understand the RT-PCR test, students may need additional background on PCR. For a brief summary of how PCR works, consider showing the *Polymerase Chain Reaction* animation. This animation does not show the first step for the RT-PCR test: reverse transcription (RT), in which the viral RNA is converted to DNA.
    - You may want to discuss the role of primers with students. As shown in the PCR animation, primers are short DNA sequences designed to bind a specific region of target DNA. In the RT-PCR test for SARS-CoV-2, the target region typically does not vary too much (or else the primers may not bind, causing false negatives) but varies enough to allow SARS-CoV-2 to be distinguished from other coronaviruses. A common target for primers is the nucleocapsid-encoding region of the viral genome; for more details on the structure of coronaviruses, refer to *Virus Explorer*.
  - Antigen tests typically detect the nucleocapsid protein, which is not present in either the antigen protein vaccines or the genetic instructions (DNA and mRNA) vaccines. Therefore, these vaccines would not be detected by the antigen tests.
  - Many antibody tests detect antibodies for the spike protein, which are produced in response to all the vaccines in the animation. However, antibody tests are not relevant to Question 20 because they should not be used to detect active viral infections, as antibodies remain long after the virus has been cleared.
  - Whether these tests detect the inactive whole virus vaccine varies. The virus is typically chemically inactivated with chemicals such as formalin and β-propiolactone. These chemicals can modify proteins, nucleotides, or both. Depending on the chemical used, certain tests may give false positives for the inactive whole virus vaccine and others may not.

TEACHING TIPS

- Consider conducting a formative assessment prior to watching the animations. Some ideas are as follows:
  - Before class, make a list of common misconceptions about SARS-CoV-2 or COVID-19 that you have become aware of in your community or seen on social media. Include several factual statements in the list as well.
    - Invite students to discuss which of these statements are accurate and which are not. Record their decisions.
    - Revisit the list after viewing the animations. Ask students if they have changed their earlier decisions about the accuracy of the statements.
  - Ask students to make a list of what they know about coronavirus infection, evolution, and detection. This can be done individually or in pairs.
    - Ask students to share one or two items from their lists with the class. Reinforce that, at this point, all ideas are to be accepted.
    - After viewing the animations, invite students to address misconceptions they had about SARS-CoV-2 and COVID-19. Ask students to discuss where some of these misconceptions may have originated.

- Consider discussing with students how they could become better informed about COVID-19 and other real-world issues and less prone to misconceptions.
  - For example, students may have seen news articles making false or misleading claims about COVID-19. Students could analyze these articles using the "Evaluating Science in the News" activity, in which they evaluate a science news article to determine whether it is trustworthy.
- Some examples in the worksheets are based on real research studies. You may wish to have students explore the original studies and data in more depth. A full list of references can be found at the end of this document. In particular:
  - In the **"Version 1"** worksheet:
    - Question 3, which describes SARS-CoV-2's ability to bind to cell receptors, is based on Wrapp et al. (2020).
    - Question 9, which describes a large deletion in some SARS-CoV-2 viruses, is based on Holland et al. (2020).
  - In the **"Version 2"** worksheet:
    - Questions 6 and 7, which discuss how the drug remdesivir could potentially treat SARS-CoV-2 infections, are based on Gordon et al. (2020).
    - Questions 10–14, which compare SARS-CoV-2 viruses in different states, use real sequence data from GenBank. GenBank links for all of these sequences are provided in the "References" at the end of this document.

STUDENT WORKSHEETS

There are two "Student Worksheets" that can be used to accompany the animations. "Version 1" was developed by a high school educator, and "Version 2" was developed by a college educator. Both worksheets were designed as learning assessments that probe students' understanding of the key concepts addressed in the animations. They can be used to assess students' understanding after watching the animations or to guide students as they watch the animations. You may modify the worksheets as needed (e.g., reducing the number of questions, adding explanations of vocabulary for English Language Learners, etc.) in order to better fit your learning objectives and your students' needs.

ANSWER KEY (VERSION 1)

PART 1: *Infection*

1. Explain how the terms COVID-19, SARS-CoV-2, and coronavirus mean different things.
   COVID-19 is a disease, and SARS-CoV-2 is the virus that causes the disease. SARS-CoV-2 is a specific type of coronavirus. Coronaviruses are a family of viruses that infect humans and other animals.
2. The diagram below represents a SARS-CoV-2 virus. In the table below the diagram, identify each of the labeled structures. Write one or two sentences describing the role of each structure in the virus's replication process.

Label	Structure	Role in replication
1	***RNA genome***	***The RNA genome carries the virus's genetic information, including genes for proteins that the virus needs to make more copies of itself.***
2	***Protein spikes***	***The protein spikes attach to specific proteins on the surface of a cell. This allows the virus to enter the cell, release its RNA genome, and use the cell's machinery to make more copies of itself.***
3	***Envelope membrane***	***The envelope gives the virus its shape and protects the virus's genome so that it can be replicated. (Since the envelope is made from cell membranes, it helps protect the virus from the immune system. You may want to explain this to students since it is not explicitly explained in the animation.)***

3. Scientists discovered that SARS-CoV-2 is 10 to 20 times more likely to bind to these receptors than the other coronavirus was. Predict how this increased chance of binding has affected SARS-CoV-2's ability to replicate.
   A coronavirus must bind to receptors on a cell in order to enter that cell. Since SARS-CoV-2 is more likely to bind to the receptors, it is more likely to enter cells and produce copies of itself. So, this increased chance of binding probably improves SARS-CoV-2's ability to replicate.

4. Each of the following statements describes a step in the replication of SARS-CoV-2. These steps are listed in random order. Read through the steps and, in the spaces provided below, list them in the order they occur in the human body.
   a. The virus releases its RNA genome into the cell.
   b. Viral RNA polymerase helps transcribe more copies of the virus's RNA.
   c. The virus binds to a receptor on a human cell's membrane.
   d. New viruses travel to the cell membrane of the infected cell and are released outside the cell.
   e. The virus's RNA is translated into proteins by the cell's ribosomes.
   Correct order: ***c, a, e, b, d***

5. As shown in the animation, SARS-CoV-2 uses the ribosomes inside human cells to translate its RNA into viral proteins. According to the animation, what is one of the viral proteins made by the ribosomes, and how does that protein help SARS-CoV-2 make more copies of itself?
   One of the viral proteins made by the ribosomes is viral RNA polymerase, which is necessary to replicate the virus's genome. The polymerase makes copies of the virus's RNA, which are used to make new viral genomes and proteins that combine to make new viruses.

PART 2: ***Evolution***

6. The SARS-CoV-2 virus has an RNA genome made of 30,000 nucleotides. Identify the four different types of nucleotides found in the SARS-CoV-2 genome.
   A, U, C, and G. (A stands for the base adenine, U for uracil, C for cytosine, and G for guanine. The names of these bases are often used as the names of the nucleotides, though they are not technically the same.)
7. Could these mutations make the virus more deadly to humans? Support your answer in one or two sentences.
   Some mutations could make the virus more deadly, if they help the virus replicate or infect cells more easily. However, other mutations might make the virus less deadly or have no effect.
8. Predict how the frequency of viruses with this deletion will change in the population over time. Use evidence to support your answer.
   This large deletion would most likely reduce or eliminate the protein's function, making it harder for the virus to escape the immune system. If so, viruses with this deletion would have a selective disadvantage (since they are more likely to be destroyed by the immune system) and would thus become less common in the population over time.
   (It is possible, although very unlikely with a deletion of this size, that the deletion could make the protein more effective at helping the virus escape the immune system. If so, the viruses with the deletion would have a selective advantage and would become more common in the population over time.)

9. How could sequencing many SARS-CoV-2 genomes be used to track how the virus has changed over time due to mutations?
   You could compare the genome sequences of SARS-CoV-2 samples taken from people in different locations over time. If there are variations among the genomes — such as more, fewer, or different nucleotides — you could track when and where these variations are present and determine when related mutations had occurred.

PART 3: ***Detection***

10. Imagine you are planning to visit a family member who is a nursing home resident. The nursing home requires that all visitors test negative for an active SARS-CoV-2 infection no more than two weeks before the visit. Which of the two tests shown above would you choose, or what other information would you want to learn before choosing? Use evidence from the table to support your answer.
Student answers will vary; be open to a range of logical responses. If students prioritize speed, they may want to get the antigen test. If they prioritize accuracy and want fewer false negatives (e.g., because they are concerned about the risk of infecting a family member), they may want to get the RT-PCR test. Students may also discuss other information they'd want to know before choosing. This could include things like the availability of the tests, where they can get tested, how much each test would cost with/without health insurance, whether there are other tests they could get, etc.

11. In addition to the tests described above, people can also get tested for the presence of SARS-CoV-2 antibodies. Explain how an individual could test negative for an active SARS-CoV-2 infection and positive for SARS-CoV-2 antibodies.
   The individual could test negative for an active infection and positive for antibodies if they were infected in the past and have since recovered. Since they have recovered, they will no longer have the virus. But they will still have antibodies from having fought the virus before.

PART 4: ***Vaccination***

12. The animation describes the following types of SARS-CoV-2 vaccines. Briefly summarize how each type of vaccine triggers an immune response. (*Hint*: Remember that the immune system responds specifically to antigens.)
    a. Inactive whole virus
       The inactive virus in the vaccine contains antigens that trigger an immune response.
    b. Antigen proteins
       The antigen proteins (e.g., spike proteins) in the vaccine trigger an immune response directly.
    c. mRNA (genetic instructions)
       The mRNA in the vaccine is translated into antigen proteins, which trigger an immune response.
    d. DNA (genetic instructions)
       The DNA in the vaccine is transcribed into mRNA. The mRNA is then translated into antigen proteins, which trigger an immune response.

13. As shown in the animation, some SARS-CoV-2 vaccines deliver mRNA for the spike protein. Mark each of the following statements about this mRNA as either true or false.

Once the mRNA has entered a person's cells, it…	True (T) or False (F)
…is translated into spike proteins.	***T***
…is integrated into the cells' DNA.	***F***
…helps trigger the production of B cells.	***T***
…is eventually eliminated from the body.	***T***

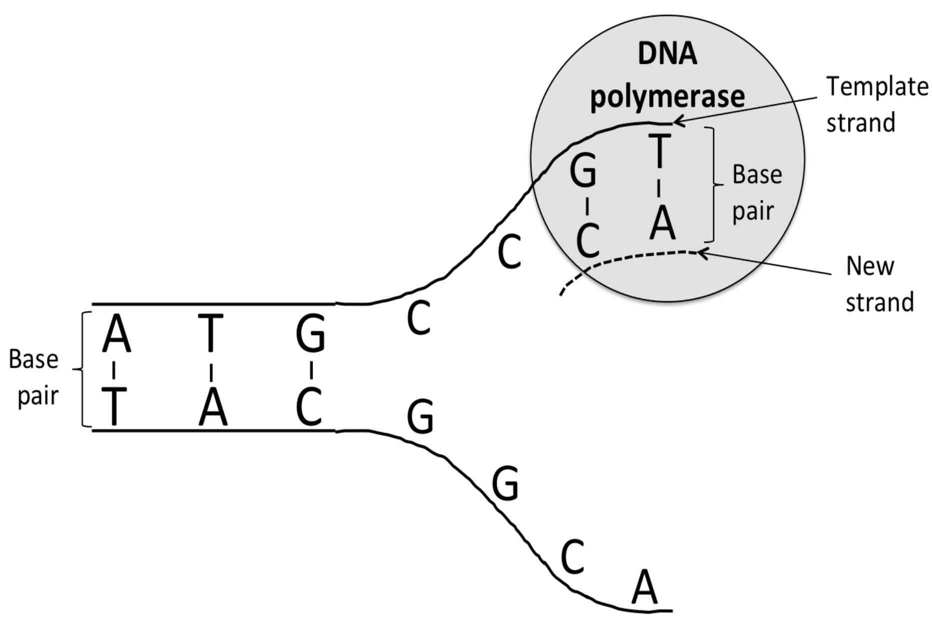
14. Why would an unvaccinated person probably need more time to recover from a SARS-CoV-2 infection than a vaccinated person would?
Assuming that the unvaccinated person hasn't had a SARS-CoV-2 infection before, they wouldn't have memory B cells or antibodies that can quickly protect them from the virus. This person would need more time for their body to produce enough B cells and antibodies to fight the virus, and they might experience more serious disease in the meantime.

15. Why might people need another SARS-CoV-2 vaccination if the SARS-CoV-2 virus mutates?***Vaccines help the body produce B cells and antibodies that target specific antigens on the virus. If the virus's antigens change due to mutations, these B cells/antibodies may no longer be able to target the virus. You may need a new vaccine, one with antigens from the mutated virus, to produce new B cells/antibodies that target the mutated virus.***

ANSWER KEY (VERSION 2)

PART 1: ***Infection***

1. Describe the shape and location of SARS-CoV-2's protein spikes.
The animation shows that the protein spikes are shaped like clubs and stick out from the virus's surface (envelope).
2. What is the function of the spike protein?
The spike proteins bind to specific proteins on the surface of a cell, allowing the virus to enter the cell.
3. Predict how mutations in the gene that encodes the spike protein might affect the types of cells the virus infects.
Mutations in the gene that encodes the spike protein could change the amino acids encoded and therefore the shape and/or chemical properties of the spike protein. These changes may increase or decrease the spike protein's ability to bind to certain cell surface proteins. Different cell types have different cell surface proteins, so changes in the spike protein could affect which cell types the virus can bind to and thus infect.
4. Draw and label a model that shows how complementary base-pairing is used to create a new strand of DNA during cellular DNA replication. Your model should include the following labels: template strand, new strand, base pair, and DNA polymerase.
Student models will vary. An example is shown below.



5. List the similarities and differences between cellular DNA replication and the RNA genome replication process used by coronaviruses.
Student answers will vary depending on what they already know about DNA replication (which is not covered in the animation). Example responses are shown below.

Similarities	
• ***occurs in cells*** • ***uses the nucleotides A, C, and G*** • ***replicates nucleic acids from "template" strands using complementary base pairing***	
Differences	
DNA replication for cells	**RNA genome replication for viruses**
• ***replicates a double-stranded molecule (DNA)*** • ***uses <u>T</u> (thymine)*** • ***uses the enzyme DNA polymerase***	• ***replicates a single-stranded molecule (RNA)*** • ***uses <u>U</u> (uracil)*** • ***uses the enzyme RNA polymerase***

6. Figure 1 shows that remdesivir "mimics" an important component of RNA replication. Which component of RNA replication has a structure similar to that of remdesivir?
In Figure 1, remdesivir appears most structurally similar to the nucleotide A.

7. Propose a hypothesis about how remdesivir might inhibit the virus's replication process.
Student hypotheses may vary, but should be stated as testable, falsifiable statements.
Example 1: Remdesivir prevents the new RNA from fully elongating.
Example 2: Remdesivir introduces mutations into the new RNA.

PART 2: ***Evolution***

8. When SARS-CoV-2 replicates in cells, mutations can occur in the virus's genome. Explain how a mutation (insertion, deletion, or substitution) in one of the virus's genes might affect a protein it encodes.
Mutations may have no effect on the protein encoded by the gene, or they may cause changes in the protein's amino acids. These amino acid changes may affect the structure, size, and function of the protein.

9. What impacts might mutations have on the transmission of the virus?
Mutations that help the virus replicate or infect cells could increase the virus's transmission. Mutations that make the virus less effective at replication or infection could decrease its transmission. Other mutations may not affect the virus's transmission at all.

10. Which of the following types of mutations is represented in Table 1?
    a. ***Substitutions (correct answer)***
    b. insertions
    c. deletions

11. At which stage of viral infection did these mutations most likely occur?
They probably occurred during the virus's genome replication process, which is prone to errors.

12. Fill in the last column of Table 1 to indicate the number of nucleotide differences between the virus from Washington and the viruses from other states.

	Position in the sequence alignment							Number of differences
State	1059	3037	14408	18060	23403	25563	28144	
Washington	C	C	C	T	A	G	C	–
Utah	C	T	T	C	G	T	T	***6***
Colorado	C	C	C	C	A	G	T	***2***
Kansas	C	T	T	C	G	G	T	***5***
California	T	T	T	C	G	T	T	***7***

13. Knowing that the virus from Washington probably arose first, make a list below predicting the order, from first to last, in which the viruses from these states arose.
    a. Washington
    b. ***Colorado***
    c. ***Kansas***
    d. ***Utah***
    e. ***California***

14. Why did you select this order?
    Mutations accumulate over time. So, the viruses with more sequence differences from the Washington virus probably arose later. (For reference, the actual collection dates for these samples were as follows. Washington: January 19, 2020; Colorado: March 7, 2020; Kansas: March 10, 2020; Utah: April 13, 2020; California: April 30, 2020.)

PART 3: ***Detection***

15. List the three types of molecules detected by the tests in the animation.
    Viral RNA, viral antigens (proteins), and antibodies

16. Imagine that three individuals get all three tests. Propose a possible explanation for each individual's test results, as shown in the following table.

Individual	RT-PCR test	Antigen test	Antibody test	Explanation
1	Positive	Positive	Negative	***This individual's RT-PCR and antigen tests are positive because they have an active infection. Their antibody test is negative because their immune system hasn't made antibodies against the virus yet.***
2	Positive	Negative	Negative	***This individual's RT-PCR test is positive because they have an active infection. Their antigen test should also be positive but produced a false negative in this case. Their antibody test is negative because their immune system hasn't made antibodies against the virus yet.*** ***(Consider discussing with students why the antigen test is more likely to produce a false negative than the RT-PCR test. The RT-PCR test is much more sensitive than the antigen test***

				because it amplifies viral RNA, which makes the virus easier to detect. The antigen test detects only the antigens/proteins that are present, so the virus must be replicating at high levels before it can be detected by the antigen test.)
3	Negativ e	Negative	Positive	***This individual's RT-PCR and antigen tests are negative because they had a past infection and recovered, so the virus is no longer in their body. Their antibody test is positive because their body still has the antibodies that were made to fight the virus.***

PART 4: ***Vaccination***

17. Antigen protein vaccines deliver the spike protein directly into the body. The DNA and mRNA vaccines, however, make the cells of the body generate the spike protein. Briefly describe how the body generates spike proteins for each vaccine type.
    a. DNA vaccines
    DNA delivered by the vaccine is transcribed into mRNA, which is translated into protein.
    b. mRNA vaccines
    mRNA delivered by the vaccine is directly translated into protein.

18. Do the genetic instructions (DNA and mRNA) vaccines contain the virus's full genome? If not, what region(s) of the genome do they contain?
    These vaccines just contain the region that encodes the spike protein.

19. Do the antigen protein vaccines contain all the proteins in the virus? If not, which protein(s) do they contain?
    These vaccines just contain the spike protein.

20. For each vaccine type in the animation, propose a test that could detect an active viral infection *without* false positives from that vaccine. (*Hint*: It may help to review the tests for active infections in the *Detection* animation. Note that there may not be a good test in every case.)

Vaccine type	**Proposed test without false positives**
Inactive whole virus	***It depends on how the virus is inactivated, but there may not be a good test in this case.***
Antigen proteins	***You could use an antigen test that detects a different viral protein than the spike protein. Or you could use an RT-PCR test that detects any part of the virus's RNA genome, since no viral RNA is delivered by this type of vaccine.***
Genetic instructions (DNA or mRNA)	***You could use an RT-PCR test that detects a different part of the virus's genome than the region that encodes the spike protein.***

REFERENCES

Astuti, Indwiani, Ysrafil. "Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response." *Diabetes & Metabolic Syndrome: Clinical Research & Reviews* 14, 4 (2020): 407–412. https://doi.org/10.1016/j.dsx.2020.04.020.

Bar-On, Yinon M., Avi Flamholz, Rob Phillips, and Ron Milo. "Science Forum: SARS-CoV-2 (COVID-19) by the numbers." *eLife* 9 (2020): e57309. https://doi.org/10.7554/elife.57309.

GenBank (for California SARS-CoV-2 isolate sequence [accession number MT460135.1]; accessed September 14, 2020). https://www.ncbi.nlm.nih.gov/nuccore/MT460135.1.

GenBank (for Colorado SARS-CoV-2 isolate sequence [accession number MT512430.1]; accessed September 14, 2020). https://www.ncbi.nlm.nih.gov/nuccore/MT512430.1.

GenBank (for Kansas SARS-CoV-2 isolate sequence [accession number MT512442.1]; accessed September 14, 2020). https://www.ncbi.nlm.nih.gov/nuccore/MT512442.1.

GenBank (for Utah SARS-CoV-2 isolate sequence [accession number MT536965.1]; accessed September 14, 2020). https://www.ncbi.nlm.nih.gov/nuccore/MT536965.1.

GenBank (for Washington SARS-CoV-2 isolate sequence [accession number MN985325.1]; accessed September 14, 2020). https://www.ncbi.nlm.nih.gov/nuccore/MN985325.1.

Gordon, Calvin J., Egor P. Tchesnokov, Emma Woolner, Jason K. Perry, Joy Y. Feng, Danielle P. Porter, and Matthias Götte. “Remdesivir Is a Direct-Acting Antiviral That Inhibits RNA-Dependent RNA Polymerase from Severe Acute Respiratory Syndrome Coronavirus 2 with High Potency.” *Journal of Biological Chemistry* 295, 20 (2020): 6785–6797. https://doi.org/10.1074/jbc.ra120.013679.

Holland, LaRinda A., Emily A. Kaelin, Rabia Maqsood, Bereket Estifanos, Lily I. Wu, Arvind Varsani, Rolf U. Halden, et al. “An 81-Nucleotide Deletion in SARS-CoV-2 ORF7a Identified from Sentinel Surveillance in Arizona (January to March 2020).” *Journal of Virology* 94, 14 (2020): 1–3. https://doi.org/10.1128/JVI.00711-20.

Lan, Jun, Jiwan Ge, Jinfang Yu, Sisi Shan, Huan Zhou, Shilong Fan, Qi Zhang, et al. “Structure of the SARS-CoV-2 Spike Receptor-Binding Domain Bound to the ACE2 Receptor.” *Nature* 581, 7807 (2020): 215–220. https://doi.org/10.1038/s41586-020-2180-5.

U.S. Food & Drug Administration. “Coronavirus Testing Basics.” Last modified July 16, 2020. https://www.fda.gov/consumers/consumer-updates/coronavirus-testing-basics.

Wrapp, Daniel, Nianshuang Wang, Kizzmekia S. Corbett, Jory A. Goldsmith, Ching-Lin Hsieh, Olubukola Abiona, Barney S. Graham, Jason S. McLellan. “Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation.” *Science* 367, 6483 (2020): 1260–1263. https://doi.org/10.1126/science.abb2507.

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