SARS-CoV-2: Understanding, Applying and Communicating Science

BIOME 2021 Working Group and Spring 2022 BioQUEST Faculty Mentoring Network

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SARS-CoV-2: Understanding, Applying and Communicating Science

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Modules: each module has sections broken up as follows:

- Suitable for high school, informal learners, and non-science major freshmen
- Suitable for non-science college major and lower-level college students
- Suitable for science major and upper-level college students

The Physiology of SARS-CoV-2 Infections: High school, informal learners, and non-science major freshmen

Learning Outcomes

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Assessment Activity: What Have You Learned?

The Physiology of SARS-CoV-2 Infections: Non-science major and lower-level college students

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Interactive Activity: Exploring the RAS System and ACE-2 Receptors
Assessment Activity: What Have You Learned?

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Background Lesson and Reading: Coronavirus Infections and the Body
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Module References

Microbiology: High school, informal learners, and non-science major freshmen

Learning Outcomes

Background Lesson and Reading: From Structure to Detection

Interactive Activity: Explore the SARS-CoV-2 Structure and Inner Workings

Assessment Activity: What Have You Learned?

Microbiology: Non-science major and lower-level college students

Learning Outcomes

Background Lesson and Reading: From Structure to Detection

Interactive Activity: Explore the SARS-CoV-2 Structure and Inner Workings

Assessment Activity: What Have You Learned?

Microbiology: Science major and upper-level college students

Learning Outcomes

Background Lesson and Reading: From Structure to Detection

Interactive Activity: Explore the SARS-CoV-2 Structure and Inner Workings

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Module references

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Learning Outcomes

Background Lesson and Reading: What is a Genetic Variant?

Interactive Activity: Explore the SARS-CoV-2 Genome

Assessment Activity: What Have You Learned?

Genetics: Variants: Science major and upper-level college students

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Background Lesson and Reading: What is a Genetic Variant?

Interactive Activity: Explore the SARS-CoV-2 Genome Variants

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Module references

Genetics: Origins: High school, informal learners, and non-science major freshmen

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Background Lesson and Reading: The Coronavirus Family

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Genetics: Origins: Science major and upper-level college students

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Module references

- **Pharmacology (in three parts): High school, informal learners, and non-science major freshmen**

  Learning Outcomes: Part 1, Part 2, Part 3

  Background Lesson and Reading: Part 1, Part 2, Part 3

  Interactive Activity: Part 1, Part 2, Part 3

  Assessment Activity: What Have You Learned? Part 1, Part 2, Part 3

- **Pharmacology (in three parts): Non-science college major and lower-level college students**

  Learning Outcomes: Part 1, Part 2, Part 3

  Background Lesson and Reading: Part 1, Part 2, Part 3

  Interactive Activity: Part 1, Part 2, Part 3

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- **Pharmacology (in three parts): Science majors and upper-level college students**

  Learning Outcomes: Part 1, Part 2, Part 3

  Background Lesson and Reading: Part 1, Part 2, Part 3

  Interactive Activity: Part 1, Part 2, Part 3

  Assessment Activity: What Have You Learned? Part 1, Part 2, Part 3

Module references

To The Instructor:

The origins of these learning modules date to Fall 2020, when instructors of the Principles of Biology course sequence at Tri-County Technical College (Pendleton, SC) decided to modify an existing HIV genetics and viral physiology lab project to address SARS-CoV-2. The goals of the original and current learning modules are to learn and apply the process of science, quantitative biology skills and thinking, science communication skills, and addressing competing scientific claims.

In the process, the current author team has expanded these modules into five modules areas: physiology, microbiology, genetics (variants and origins), and pharmacology. Each module is subdivided into blue/gray (suitable for high school and community science major learners), yellow (freshman and non-science majors), and red (suitable for college science major learners) sections. As an instructor, you are free to make use of any, all, or pieces of the material, and should use the hyperlinking in the Table of Contents to help you direct your students to the appropriate material. Each module area and subsection has its own citations section, should you wish to have your students do further research, apart from the provided material.

To The Learner:

The origins of these learning modules date to Fall 2020, when instructors of the Principles of Biology course sequence at Tri-County Technical College (Pendleton, SC) decided to modify an existing HIV genetics and viral physiology lab project to address SARS-CoV-2. The goals of the original and current learning modules are to learn and apply the process of science, quantitative biology skills and thinking, science communication skills, and addressing competing scientific claims.

There are four modules: physiology, microbiology, genetics, and pharmacology. Each module area is subdivided into blue/gray (suitable for high school and community science major learners), yellow (freshman and non-science majors), and red (suitable for college science major learners) sections. Your instructor will use the hyperlinking in the Table of Contents to help you to the learning material they have selected. You are welcome to do further exploration on your own, using the citations we have provided in each section as a jumping off point. Please feel free to let us know if there is material you would like to see added, or if something seemed confusing to you.

The Physiology of SARS-CoV-2 Infections – High School, Informal Learners, and Non-Science Major College Freshman

Learning Outcomes:

- Understand the history of coronavirus infections in animals, including humans.
- Determine how coronaviruses invade animal cells.
- Understand why researchers target spike proteins, receptor binding proteins and tissue receptors.

Background Lesson and Reading: Making the Animal to Human Jump

Various coronaviruses have made the leap, probably many times, from non-human animals to humans. Host species have included civet cats, camels, bats and pangolin (Sallard et al. 2021; Carlson et al. 2021). The 2002 – 2003 SARS-CoV, and the 2012 MERS-CoV, epidemics (OMB#: 0925-0668 2020; O'Sullivan 2021) provided something of a wakeup call to the dangers posed by two clades of coronaviruses (the beta and delta clades in particular; Aurora et al. 2020).

Early in the pandemic, it became clear that COVID-19 impacts the respiratory system most acutely, followed by the cardiovascular and nervous systems, and then by other body systems (Yuki et al. 2020). In all cases, acute inflammation and subsequent symptoms appear to be the major cause of symptomatic illness. Patients experience mild to moderate fevers, dry coughs, weakness, dizziness, vomiting, diarrhea, and mental confusion. Those general symptoms can rapidly progress significant hypoxia (low blood oxygen levels) and acute respiratory distress syndrome (ARDS), cardiovascular failure and/or stroke (Bohn et al. 2020).

You may have heard claims that SARS-CoV-2 (the coronavirus that causes COVID-19) was either manufactured in a lab, and then accidentally or deliberately released, or that a wild strain was being studied in a lab from where it was

released. There are ways to test those hypotheses against the hypothesis that one of many accidental zoonotic transfers of SARS-CoV-2 from a bat colony mutated enough to become highly transmissible. You will learn to test those hypotheses in the Genetics Module.

Explore the following links, and answer the accompanying questions, before proceeding to the interactive activity:

1. Why is SARS-CoV-2 so good at infecting human cells: https://youtu.be/v0lXtzfSPbw

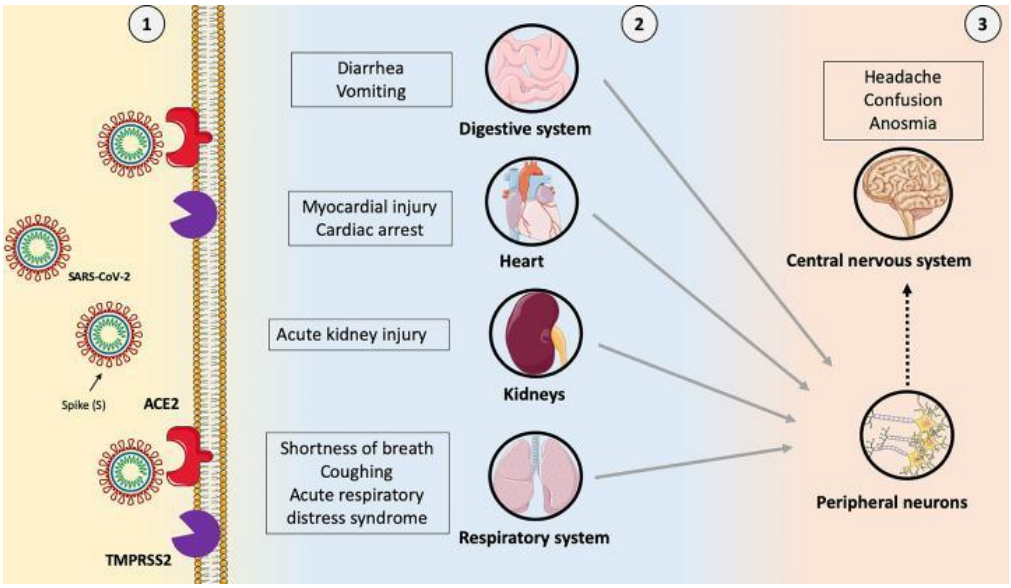
2. Explore the omicron variant mutations: https://www.washingtonpost.com/health/2021/12/16/omicron-variant-mutations-covid/

3. COVID-19 and respiratory physiology: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8279806/; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7453378/

4. COVID-19 and the heart: https://www.nhlbi.nih.gov/coronavirus/heart; https://link.springer.com/article/10.1007/s11936-022-00964-3

Also, explore the image below to see how COVID-19 effects various organs and tissues in the human body.

Figure 1: Impacts from COVID-19 on the body



(Battagello et al. 2020)

Interactive Activity: Patient Case Study

Case 1: Greg and Sandra

Sandra and Greg are active, late middle-aged, middle-class adults who are retired. Every year, they take a two-three month camping trip in their RV, exploring national parks and visiting far-flung family members. Their son lives nearby, and they have two young grandchildren they love visiting with. Greg is very physically active, loves to boat and fish, ride his motorcycle, and hiking and camping.

Both Sandra and Greg are healthy, but Greg has asthma and is an ex- smoker, and regularly uses a bronchodilator with fluticasone propionate. During the summer of 2020, Sandra and Greg were carefully staying relatively isolated, avoiding church, eating out, and other large social gatherings. They continued to babysit their grandson, and meet with a few people outside. Their son and his wife work in high contact fields, but were as careful as possible.

In July, Sandra started feeling like she had a 'summer cold.' She developed a mild fever and a cough, and tested positive for COVID-19. Since her symptoms seemed mild, Greg went fishing for the weekend. Upon return, he too developed a cough and cold-like symptoms. Greg's symptoms rapidly progressed, and after a week of trying to manage them, he too tested positive. Greg found he was having increasing difficulty breathing, and went to the ER when his heart started racing. By this time, he had a persistent high fever, cough, rapid heart rate, and couldn't catch his breath. He tested positive for COVID-19, and was told his blood oxygen saturation was at 84% and that he was experiencing cardiac arrhythmias. He was given high-dose steroids, and told that if his oxygen saturation didn't recover, he would be intubated and put on a ventilator. He and Sandra contacted their priest, believing he would not survive.

The high dose steroids did help Greg's respiratory symptoms to abate, and his cardiac symptoms slowly disappeared. He had persistent ground glass opacity (GGO; areas in the lungs that show up as gray in an X-ray, that may be filled with fluid and pus) and pneumonia for approximately six months after testing negative.

Once vaccines became available, both Greg and Sandra were vaccinated, and accepted a booster vaccine when that was authorized. Neither has tested positive for COVID-19 subsequently.

Case Study Questions:

1. Why do you think Greg had a more severe case of COVID-19?
2. Why do you think Sandra and Greg became ill, when they were being so careful?
3. Why did the hospital prescribe high-dose steroids for Greg?
4. Why is GGO a frequent effect of COVID-19?
5. Why would GGO show up on an X-Ray as gray patches in the lungs.

Assessment Activity: What Have You Learned?

1. Select the three best ways to slow the spread of COVID-19:

a. masking b. distancing c. 'megadosing' vitamin C

c. meditation d. essential oils e. vaccination

2. In your own words, explain why blood oxygen levels are important to health.

3. What is a spike protein?

a. It's a protein used to spike drinks

b. It's a protein shaped like a spike, that punctures cell membranes.

c. It's a protein that helps a virus gain entry into a host cell.

4. Name three key differences between the **symptoms** of the prior strains of COVID-19 and the omicron strain.

The Physiology of SARS-CoV-2 Infections – Non-Science Major and Lower Level College Students

Learning Outcomes:

- Understand the RAS system and the importance of ACE-2 receptors within that system.
- Integrate understanding of how the RAS system operates, the distribution of ACE-2 receptors in the human body, and why that distribution pattern allows a wide-array of COVID-19 symptoms.
- Understand the importance of form-function relationships in the binding of SARS-CoV-2 spike proteins to ACE-2 receptors.

Background Lesson and Reading: Coronavirus Infections and the Body

As with all animal viruses, SARS-CoV-2 has proteins in its envelope or coat (see video: https://youtu.be/v0lXtzfSPbw). Some of those proteins bind directly receptors in animal tissues called the angiotensin-converting enzyme-2 (ACE-2) receptors. Those receptors are like gates into the cell they're found on, and ACE-2 receptors are found on almost every body tissue. That's part of the reason COVID-19 has so many different infectious processes – it really depends on where the greatest 'load' of viruses binding to receptors is. The main protein that allows coronaviruses to attach to ACE-2 receptors is called the "spike protein." That protein acts like a key in a lock, allowing it enter a cell. SARS-CoV-2 has a particularly well-fitted key, so it binds to the ACE-2 lock very, very well, allowing rapid entry into cells with the receptor.

Explore the following two links before proceeding:

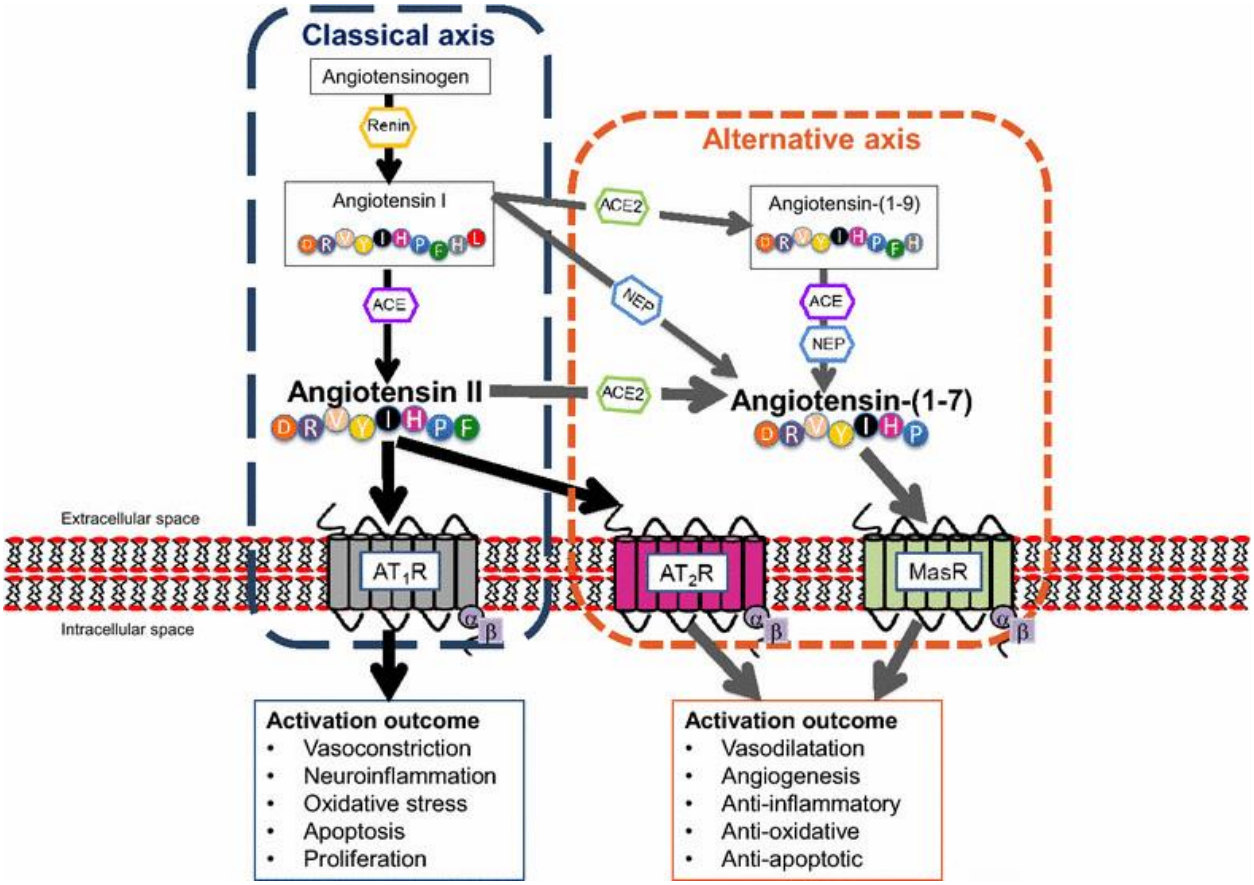
1. Simulator: https://s3.us-west-1.amazonaws.com/tfs.dig/TF_CarsCov2_variant_viewer/index.html

2. https://www.news-medical.net/health/How-does-a-SARS-CoV-2-Virion-Bind-to-ACE2.aspx

3. https://www.researchgate.net/publication/308944248_Therapeutic_potential_of_the_renin_angiotensin_system_in_ischaemic_stroke

Also, explore the image below to better understand how SARS-Cov-2 interacts with the RAS system.

Figure 1: Alternative RAS pathways



(https://www.researchgate.net/publication/308944248_Therapeutic_potential_of_the_renin_angiotensin_system_in_ischaemic_stroke)

Interactive Activity: Exploring the RAS System and ACE-2 Receptors

Explore the image in the prior paragraph, and the interactive image below to answer the questions.

RAS System and ACE-2 Receptor Questions:

1. Which tissues are especially vulnerable to COVID-19?

2. Why does binding of SARS-CoV-2 to ACE-2 receptors lead to the tissues in Question 1 being vulnerable?

3. What are the most important clinical signs of COVID-19?

4. Using the RAS system and ACE receptor pathways in the preceding figures, explain why those clinical signs of COVID-19 (Question 3) occur.

Assessment Activity: What Have You Learned?

1. Explain the differences between the ACE-1 and ACE-2 receptor pathways.

2. What roles do the ACE-1 and ACE-2 receptor pathways play in the RAS system?

3. Review the Case Study in the section of the physiological module. What part of the RAS system was being activated in Greg's infection course? Which ACE receptor(s) did the virus target in him, and how do you know? What, specifically, caused the GGO he experienced for months after he no longer tested positive?

The Physiology of SARS-CoV-2 Infections – Science-Major and Upper Level College Students

Learning Outcomes:

- Understand the RAS system and the importance of ACE-2 receptors within that system.
- Integrate understanding of how the RAS system operates, the distribution of ACE-2 receptors in the human body, and why that distribution pattern allows a wide-array of COVID-19 symptoms.
- Understand the importance of form-function relationships in the binding of SARS-CoV-2 spike proteins to ACE-2 receptors.
- Understand how cleavage of the receptor binding protein enables a tighter fit of the spike protein to the ACE-2 receptor.

Background Lesson and Reading: Coronavirus Infections and the Body

As with all animal viruses, SARS-CoV-2 has proteins in its envelope or coat (see video: https://youtu.be/v0lXtzfSPbw). Some of those proteins bind directly receptors in animal tissues called the angiotensin-converting enzyme-2 (ACE-2) receptors. Those receptors are like gates into the cell they're found on, and ACE-2 receptors are found on almost every body tissue. That's part of the reason COVID-19 has so many different infectious processes – it really depends on where the greatest 'load' of viruses binding to receptors is. The main protein that allows coronaviruses to attach to ACE-2 receptors is called the "spike protein." That protein acts like a key in a lock, allowing it enter a cell. SARS-CoV-2 has a particularly well-fitted key, so it binds to the ACE-2 lock very, very well, allowing rapid entry into cells with the receptor.

ACE-2 receptors are the key receptors in the renin-angiotensin system, so researchers are working on ACE-2 receptor blockers as a possible means of

treating the symptoms of COVID-19 (Flacco et al. 2020). We don't have enough data yet to know if this treatment might work, but it's an interesting approach. While both Merck and Phizer have released anti-viral pills to treat the symptoms of COVID-19 when it's caught early enough, there really is no substitute for vaccination, which helps to train T and B cells on proper responses to novel antigens.

Explore the following links before proceeding:

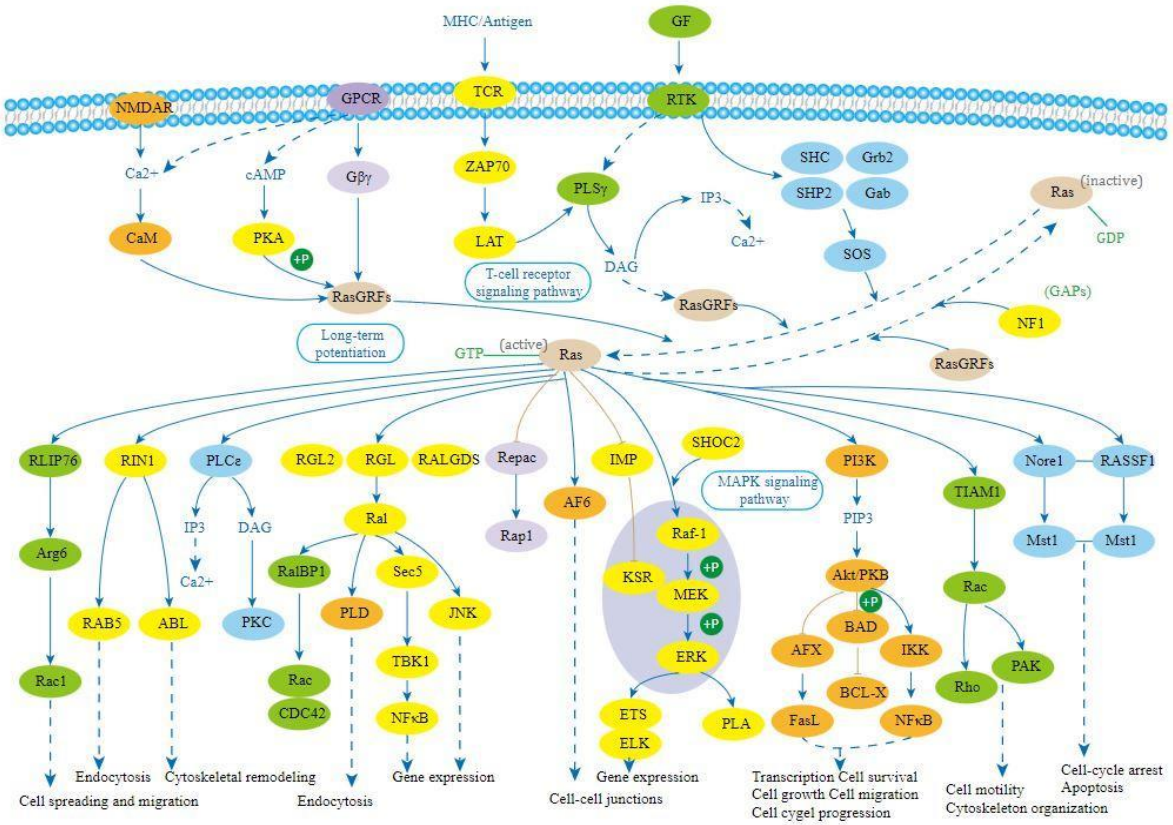
1. Simulator: https://s3.us-west-1.amazonaws.com/tfs.dig/TF_CarsCov2_variant_viewer/index.html

2. Pathophysiology: https://youtu.be/xzacuQtbOg8

3. RAS system genes and proteins: https://www.addgene.org/cancer/ras-pathway/

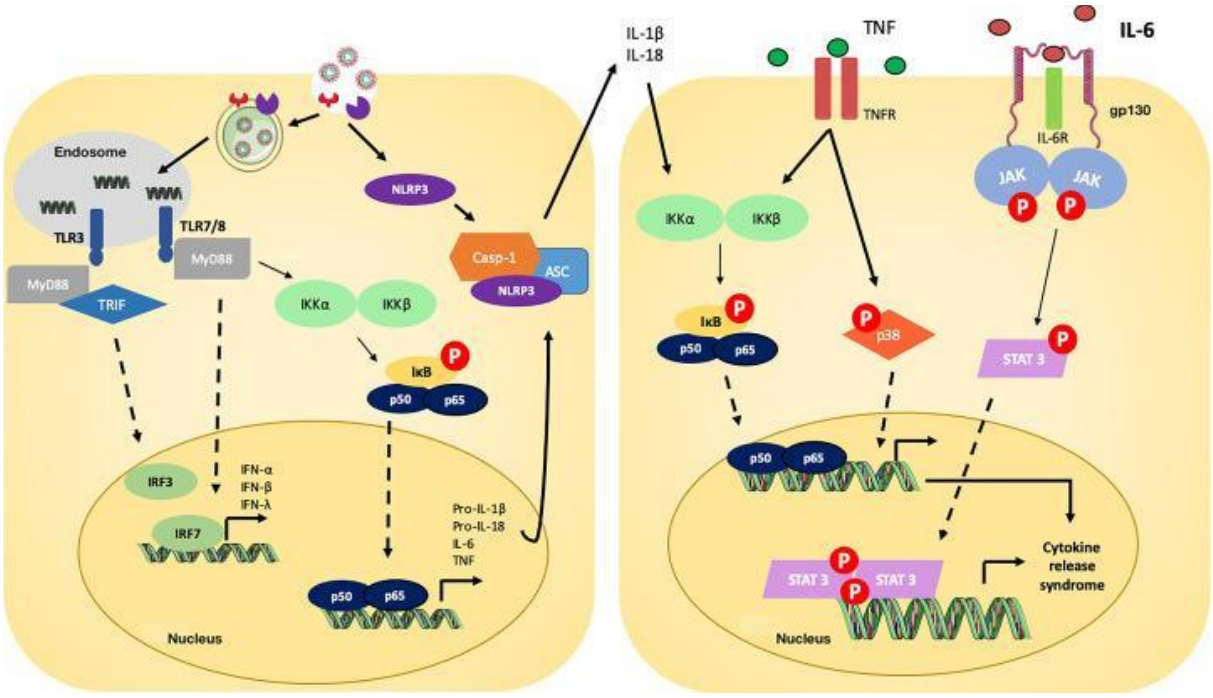
Also, explore the images below to better understand how SARS-Cov-2 interacts with the RAS system.

Figure 1: T-cell signaling and RAS pathways



(https://www.cusabio.com/pathway/Ras-signaling-pathway-1.html)

Figure 2: RAS pathway control of genetic expression



(Battagello et al. 2020)

Interactive Activity: RAS Pathway Sorting Game

Sort the pathway between infection of SARS-CoV-2, the RAS system, and the immune response. https://puzzel.org/en/reorder/play?p=-MyTmdFJZm8bz14DVJt7

RAS Pathway Questions:

1. Which tissues are especially vulnerable to COVID-19?

2. Why does binding of SARS-CoV-2 to ACE-2 receptors lead to the tissues in Question 1 being vulnerable?

3. What are the most important clinical signs of COVID-19?

4. Using the RAS system and ACE receptor pathways in the preceding figures, explain why those clinical signs of COVID-19 (Question 3) occur.

Assessment Activity: What Have You Learned?

1. Using the third link in the background system, lay out the key genes and proteins involved in the ACE pathways targeted by SARS-CoV-2. Explain the roles those genes and proteins play in the course of the infection for at least two key tissues or organs.

2. After exploring at least three scientific literature sources, describe what do you think is the leading hypothesis for how SARS-CoV-2 entered human populations? Justify your answer from your reading the literature, and cite your sources.

References:

Ye C, Cai S, Shen G, Guan H, Zhou L, Hu Y, Tu W, Chen Y, Yu Y, Wu X, Chen Y, Zhong J, Dong L. 2020. Clinical features of rheumatic patients infected with COVID-19 in Wuhan, China. Ann. Rheum Dis. 79: 1007-1013.

Sallard E, Halloy J, Casane D, Decroly E, van Helden J. 2021. Tracing the origins of SARS-CoV-2 in coronavirus phylogenies: a review. Environ. Chem. Lett. 19(2): 769-785.

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Arora P, Jafferany M, Lotti T, Sadoughifar R, Goldust M. 2020. Learning from history: coronavirus outbreaks in the past. Dermatologic Therapy. 33(4): e13343.

Yuki K, Fujiogi M, Koutsogiannaki S. 2020. COVID-19 pathophysiology: a review. Clin. Immunol. 215: 108427.

Bohn MK, Hall A, Sepiashivili, L, Jung B, Steele S, Adeli K. 2020. Pathophysiology of COVID-19: mechanisms underlying disease severity and progression. Physiology. 35: 5.

Yan R, Zhang Y, Yaning L, Xia L, Guo Y, Zhou Q. 2020. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science. 367(6485): 1444-1448.

The Microbiology of SARS-CoV-2 – High School, Informal Learners, and Non-Science Major College Freshman

Learning Outcomes:

- Understand what are coronaviruses and the role of SARS-CoV-2
- Appraise how SARS-CoV-2 is built and how it infects us
- Comprehend the reasons why SARS-CoV-2 is so contagious
- Explain how SARS-CoV-2 can survive among us
- Understand how we can detect the virus

Background Lesson and Reading: From Structure to Detection

Taxonomy: Coronavirus disease (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) – so the former designation represents a disease, and the latter is a causative virus. This virus belongs to the big coronavirus family, which is a group that includes common cold coronaviruses, but also very dangerous SARS-CoV and MERS-CoV – two other coronaviruses that can seriously injure human lungs. Viruses have genetic material surrounded by a protein coat, and in the case of SARS-CoV-2, this genetic material is RNA (while some other viruses have DNA). Can you remember what RNA is and why it is different from DNA?

Structure: Coronaviruses are small viruses that can only be seen with an electron microscope, and they have an envelope with a characteristic crown-like appearance visible under an electron microscope, which is largely due to the presence of characteristic molecules known as spike proteins. This is the reason behind the name for the whole viral family (‘corona’ in Latin translates as ‘the crown’). In any case, these structures are crucial for viral entry into our cells, and

together with other proteins, they also help the virus to develop affinity for human cells. These proteins are coded on viral genome, which also codes for other proteins, such as those found in the membrane or near the RNA. One outcome of this module is to get acquainted with the simplified diagram of coronavirus particle structure (Figure 1), which is important to adequately recognize in the era of pervasive pandemic infographics. Here on this Figure 1 we can clearly see spike proteins giving the specific viral crown appearance.

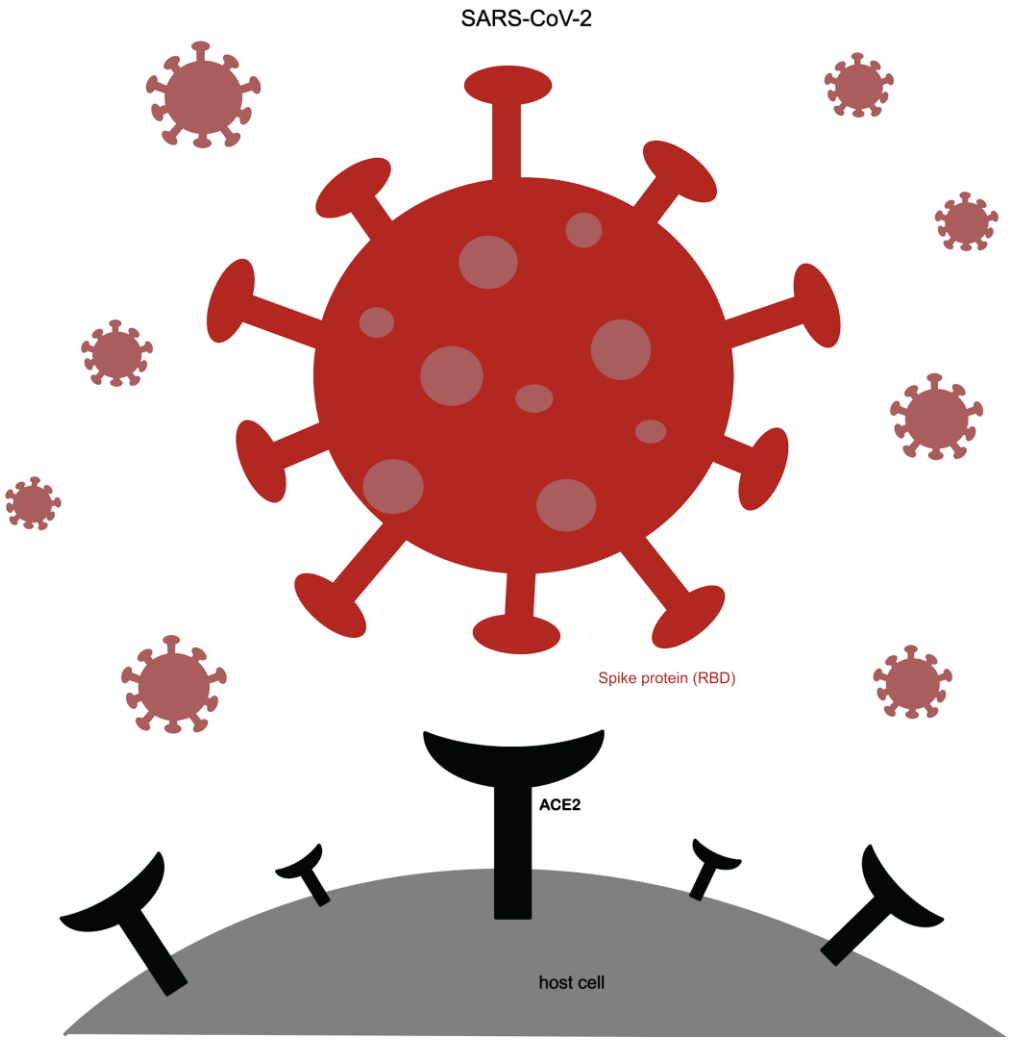


Figure 1: Model of SARS-CoV-2 virus binding to host cell

Cell entry and viral replication: Viruses have to infect a living cell and hijack its structures to make copies of themselves. As SARS-CoV-2 gains access to our cells very easily, that makes it very infectious and transmissible. Spike proteins on the surface of viruses can readily attach to specific molecules on the surface of our cells (Figure 1), and so SARS-CoV-2 can insert its genetic material into the cell. Once inside, the virus can replicate and make new viral particles, before transporting them to the surface of our cells, as explained in the Physiology module. The newly formed infectious viral particles are then released from the host cell and can infect other individuals.

Resistance to physical and chemical agents: In the absence of any ventilation, SARS-CoV-2 can survive in very small liquid droplets (known as aerosols) for three hours. Viral particles are most stable on stainless steel and plastic; furthermore, the virus is much more stable on smooth surfaces like glass, steel and plastic (where it can survive several days) when compared to rough surfaces like paper, wood and cloth (where it can survive for several hours). SARS-CoV-2 and other coronaviruses can be effectively inactivated by lipid solvents and common disinfectants. Soaps can dissolve various viruses, and in the same manner they can also inactivate SARS-CoV-2.

Transmission: SARS-CoV-2 is transmitted from human to human by infectious droplets, especially during prolonged close contact (most commonly in the household). Transmission may also occur indirectly via contaminated surfaces or objects likely to carry infectious particles, even though that risk is considered to be low. Inhalation of very fine respiratory droplets and aerosol particles is also a way of viral transmission. Lung cells are the prime target of the virus, and this is the ideal point to consider the size difference between a SARS-CoV-2 virus particle and the lung cells it infects, as 800 viral particles can be lined up across the diameter of a single lung cell. Moreover, a single viral particle that infects a single lung cell can release a thousand replicated viral particles over the course of ten hours, by exponential multiplication. Each of these particles can infect other lung cells, or be breathed out through the lung into the air in either droplets (after cough or sneeze)

or microdroplets (even when just breathing). Do you understand the concept of exponential growth and what does it mean?

Viral detection: The so-called 'gold standard' for SARS-CoV-2 diagnostics are molecular methods, which aim to detect genetic material in SARS-CoV-2 viral particles from upper respiratory tract samples by using swabs, but in some instances also lower respiratory tract samples. You will often hear the method of detection abbreviated as polymerase chain reaction or PCR, and this is used to very reliably detect the active presence of the virus in our body. As already mentioned, first-line testing usually entails upper respiratory tract samples, which are much easier to take and come with lower viral transmission risk; on the other hand, lower respiratory tract samples are reserved for selected patients in hospitals, where the clinical presentation of COVID-19 is usually more severe. There are also test that measure the immune response of the organism that creates antibodies directed against SARS-CoV-2 proteins, for which a blood sample is needed. These tests are called serology assays, and they are sometimes also used to try and quantify our response against the virus.

Finally, rapid tests have been developed with the underlying idea of a point-of-care approach, offering rapid results within 10-30 minutes. There are currently rapid tests for both the detection of the virus (so-called rapid antigen tests) and the detection of antibodies, i.e. the response from our organism under attack (so-called rapid antibody tests). These tests provide prompt, but only qualitative information (for example, whether the virus or antibodies are present or not).

Interactive Activity: Explore the SARS-CoV-2 Structure and Inner Workings

An interactive graphical story: Inside the Coronavirus

What scientists know about the inner workings of the pathogen that has infected the world by Scientific American.

Link: https://www.scientificamerican.com/interactive/inside-the-coronavirus/

Coronavirus Anatomy Explained: Science, Simplified

An animated look at the inner workings of the coronavirus that causes COVID-19. Illustrated by a Scripps Research scientist, this installment of Science, Simplified gives an overview of the key elements of SARS-CoV-2.

Link: https://www.youtube.com/watch?v=8hgc2iZflTI

World Health Organization (WHO): How COVID-19 is transmitted

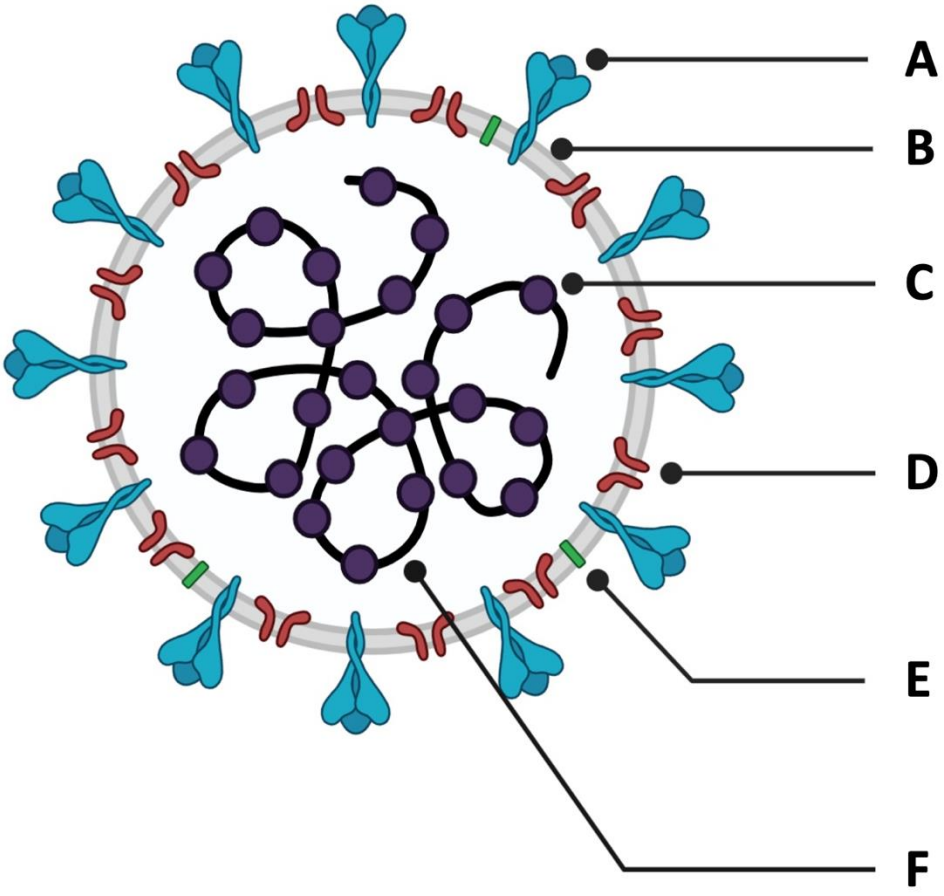
An animated story by the WHO explaining how SARS-CoV-2 spreads mainly between people in close contact with each other. It spreads most easily in crowded settings, closed spaces with poor ventilation or through prolonged contact with an infected person.

Link: https://www.youtube.com/watch?v=oqFn6AHoJZQ

Assessment Activity: What Have You Learned?

1. Explain how the designations COVID-19, SARS-CoV-2 and coronavirus mean different things, and why is it important to be precise when using these different designations?

2. The diagram below represents a SARS-CoV-2 virus. Based on the text and image above, try to identify where the spike glycoprotein (S protein) is situated in this scheme, and its role in causing the disease.



3. In a hypothetical example, how many particles would be released if each of the 1,000 particles that originally entered the lungs continue to infect cells and create 1,000 more particles each in 10 additional hours (e.g., by 20 hours)? What happens in the next ten hours? Calculate and fill in the

number or particles released at 30, 40 and 50 hours. On the bottom row, change the numbers to exponential notation.

Time from infection	10 hours	20 hours	30 hours	40 hours	50 hours
SARS-CoV-2 particles released	1,000	1,000,000			
Exponential	10^3				

4. Imagine you plan 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 tests explained above would you choose – molecular ones that detect viral genetic material or serological ones that detect antibodies? Use the explanation in the text to support your answer.

The Microbiology of SARS-CoV-2 – Non-Science Major and Upper-Level College Students

Learning Outcomes:

- Understand classification of coronaviruses and compare different coronaviruses
- Appraise structure of SARS-CoV-2 and other coronaviruses and understand how it infects the cell
- Comprehend the reasons why SARS-CoV-2 is so infectious and can easily spread
- Explain how SARS-CoV-2 can survive in the presence of various agents that can inactivate it
- Understand how we can detect the virus

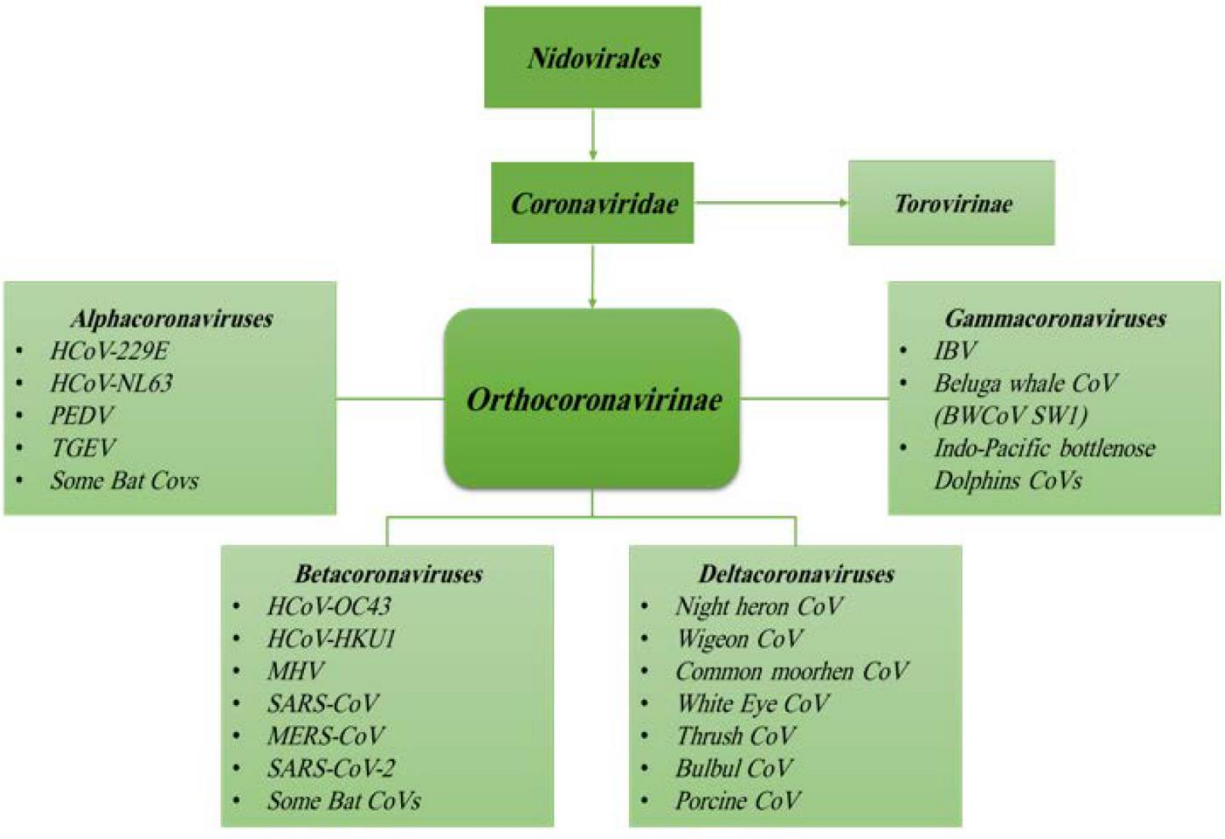
Background Lesson and Reading: From Structure to Detection

Taxonomy: Coronavirus disease (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) – so the former is a disease, and the latter is a causative virus. This virus belongs to the *Coronaviridae* family. There are four subgroups of the coronavirus family, and SARS-CoV-2 is actually a beta-coronavirus, which is a group that also includes SARS-CoV and MERS-CoV, two other notable coronaviruses that can injure human lungs (Figure 1). SARS-CoV-2 is most closely related to SARS-CoV, and all coronaviruses have a specific RNA genome. Do you know what the difference between viruses with RNA and DNA genome is?

Structure: Coronaviruses are small (between 60 and 120 nanometers in diameter) viruses with an envelope that have a characteristic crown-like appearance visible under an electron microscope, which is largely due to the presence of characteristic spike glycoproteins – these are specific molecules that contain a protein plus a

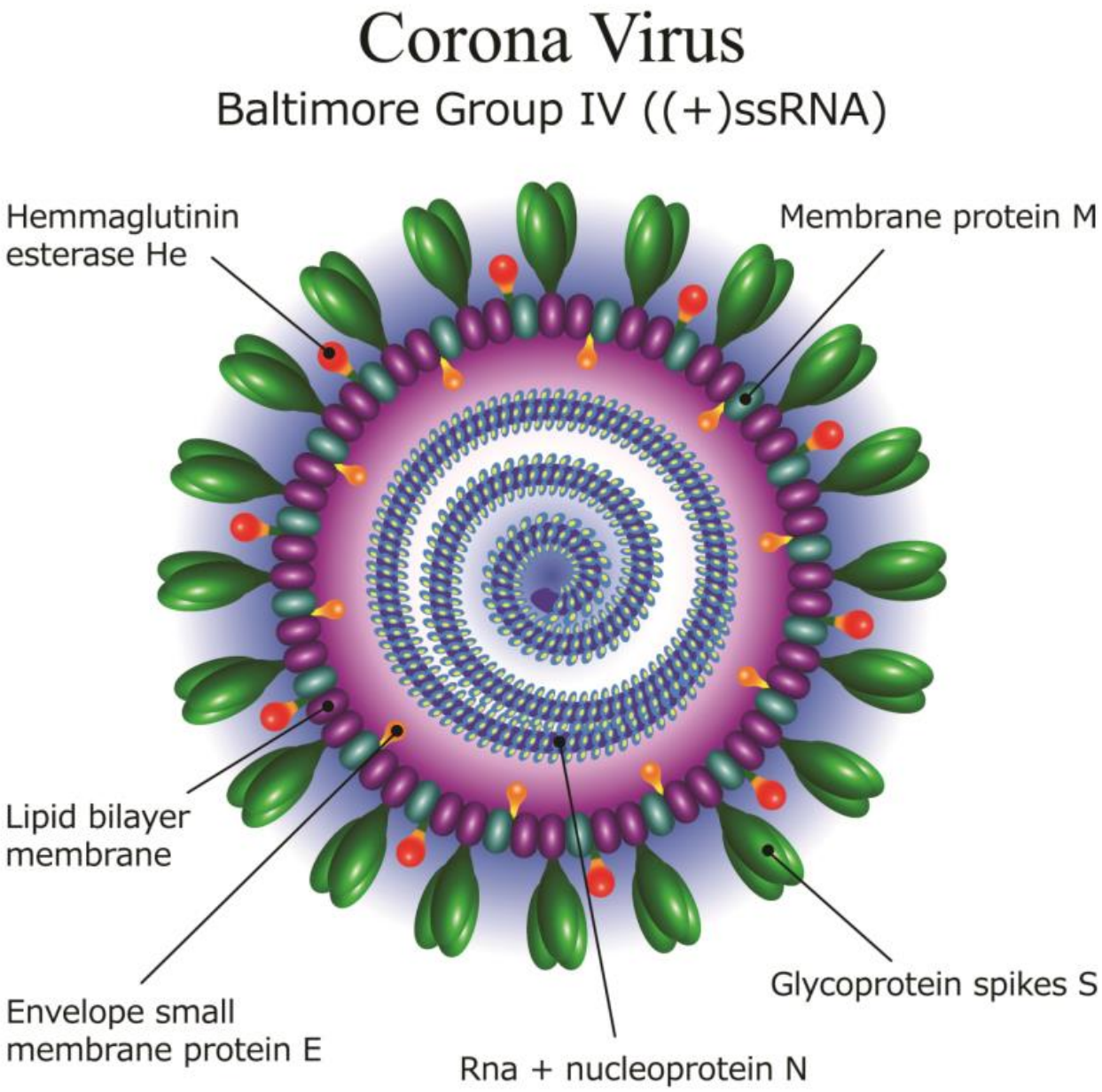
carbohydrate. This is actually the reason behind the name for the whole viral family ('corona' in Latin translates as 'the crown'). These proteins are coded on large RNA genomes characteristic for coronaviruses, which also codes for membrane, envelope and nucleocapsid proteins. There are also some proteins (and one of the most important ones is hemmaglutinin esterase) that can influence the affinity of SARS-CoV-2 for human cells, which is known as viral tropism. However, as we will show in continuation of this text, spike glycoprotein (also known as S protein) is the most important one for cell entry and subsequent infection. One outcome of this module is to get acquainted with the diagram of coronavirus particle structure (Figure 2), which is important to recognize in the era of pervasive pandemic infographics.

Figure 1: Coronaviruses with notable respiratory effects



(source: Hozhabri H et al. doi: 10.3390/ijerph17165648)

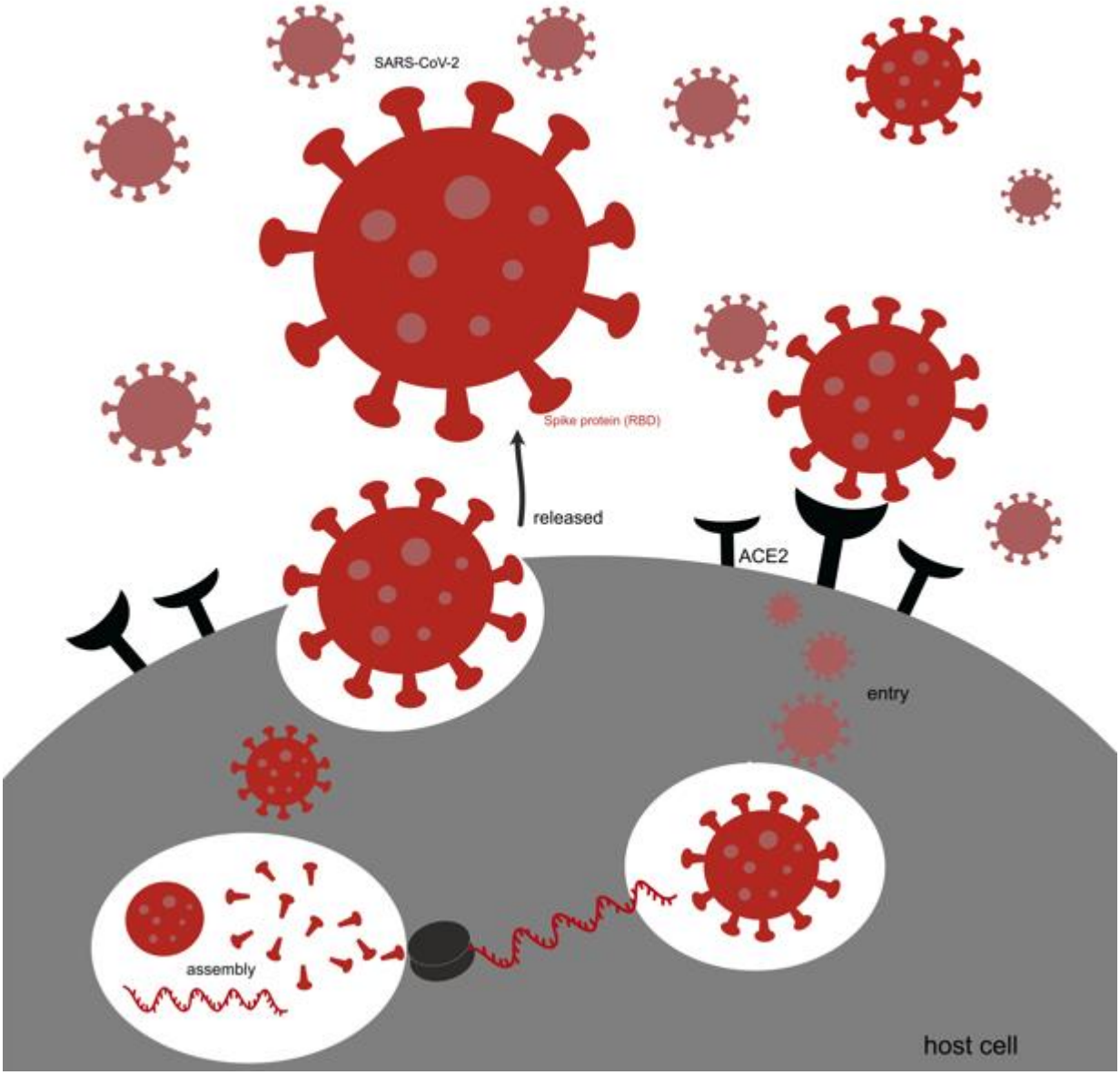
Figure 2: General structure of a coronavirus particle



Cell entry and viral replication: Why is SARS-CoV-2 so much more infectious than SARS-CoV or MERS-CoV? One possible reason may be in how this virus can gain access to host cells. Viral binding to the cells occurs via the interaction of the spike glycoprotein of SARS-CoV-2 with Angiotensin-converting enzyme 2 (ACE2), which is a specific protein on the surface of the cell that acts like a receptor (primarily expressed on lung cells). After binding to the receptor, the virus must gain access to the host cell which, for coronaviruses, usually entails splitting of the S protein and then fusion of the viral and cellular membranes. The next steps leading to viral replication would involve viral RNA translation, assembly of specific viral complexes, viral RNA synthesis, assembly of viral particles, and transport of these to the cell surface inside vesicles, as explained in the Physiology module. The newly formed infectious viral particles are then released from the host cell. Although the original SARS-CoV uses the same cell receptor, SARS-CoV-2 goes through the infectious process much quicker. The summary of this infectious cycle can be seen on Figure 3.

Resistance to physical and chemical agents: In the absence of any ventilation, SARS-CoV-2 can survive in very small liquid droplets (known as aerosols) for 3 hours. Viral particles are most stable on stainless steel and plastic, where virus can survive for up to 72 hours in the absence of disinfection. Furthermore, SARS-CoV-2 is more stable on smooth surfaces like glass, steel and plastic (several days) than on rough surfaces like paper, wood and cloth (several hours). Like other coronaviruses, SARS-CoV-2 is very stable at 4 °C but sensitive to ultraviolet rays and heat. In addition, these viruses can be effectively inactivated by lipid solvents and common disinfectants – including ether, chlorine-containing disinfectant, ethanol and chloroform. Soaps can dissolve various viruses, thus they can also inactivate SARS-CoV-2.

Figure 3: Summary of the infectious cycle of SARS-CoV-2



Transmission: SARS-CoV-2 is transmitted from human to human by infectious droplets. More specifically, contact tracing studies show that prolonged close contact is the main risk factor for transmission and that the risk of infection is much higher in household contacts compared to non-household contacts. Transmission may also occur indirectly via contaminated surfaces or fomites (objects or materials likely to carry infectious particles), even though that risk is considered to be low. Moreover, SARS-CoV-2 genetic material has not only been found in upper respiratory tract secretions, but also in other body fluids such as blood, feces and (in rare instances) urine. The potential of long-range airborne transmission of SARS-CoV-2 is also widely accepted, and the inhalation of very fine respiratory droplets and aerosol particles is also a way of viral transmission. Lung cells are the prime target of the virus, and this is the ideal point to consider the size difference between a SARS-CoV-2 virus particle and the cell it infects (which are basically type 1 alveolar epithelial cells located deep in the lung). Those lung cells are around 80 µm, so you could actually line up 800 viral particles across the diameter of a single lung cell. Furthermore, a single viral particle that infects a single lung cell can release 1,000 replicated viral particles over the ten hours, by exponential multiplication. Each of these particles can infect other lung cells, or be breathed out through the lung into the air in either droplets (after cough or sneeze) or microdroplets (even when just breathing). Do you remember the concept of exponential growth and what does it mean?

Viral detection: The so-called 'gold standard' for SARS-CoV-2 diagnostics are molecular methods, which primarily involve polymerase chain reaction (PCR). These assays seek to qualitatively detect nucleic acid from SARS-CoV-2 on upper respiratory tract samples (such as samples from nasopharynx, pharynx or nose), but in some instances also lower respiratory tract samples. First line testing usually entails upper respiratory tract samples, which are much easier to take and come with lower viral transmission risk; on the other hand, lower respiratory tract samples are reserved for selected patients in hospitals, where the clinical presentation of COVID-19 is usually more severe. Immunological assays, or serology tests, have been developed to measure the immune response of the organism that creates antibodies directed against SARS-CoV-2 proteins, for which a blood sample

is needed. Currently available assays target the main immunogenic coronavirus proteins, such as the aforementioned S protein or nucleoprotein.

Rapid tests have been developed with the underlying idea of a point-of-care approach, offering rapid results (within 10-30 minutes). There are currently rapid tests for both the detection of antigens and the detection of antibodies. These tests provide prompt, but only qualitative information (for example, whether the virus or antibodies are present or not). The table below summarizes and compares some aspects of these tests.

Table 1: Test methods for COVID-19

Type of test	**Testing for the presence of**	**Test accuracy**	**Time to obtain results**
PCR test	Pieces of the viral RNA genome	Infection is almost always detected; test usually does not have to be repeated.	From one day to one week
Antigen test	Parts of viral proteins (also known as antigens)	Sometimes the infection is missed, so other test may have to be used to confirm negative results.	One hour or less

Interactive Activity: Explore the SARS-CoV-2 Structure and Inner Workings

An interactive graphical story: Inside the Coronavirus

What scientists know about the inner workings of the pathogen that has infected the world by Scientific American.

Link: https://www.scientificamerican.com/interactive/inside-the-coronavirus/

Coronavirus Anatomy Explained: Science, Simplified

An animated look at the inner workings of the coronavirus that causes COVID-19. Illustrated by a Scripps Research scientist, this installment of Science, Simplified gives an overview of the key elements of SARS-CoV-2.

Link: https://www.youtube.com/watch?v=8hgc2iZflTI

World Health Organization (WHO): How COVID-19 is transmitted

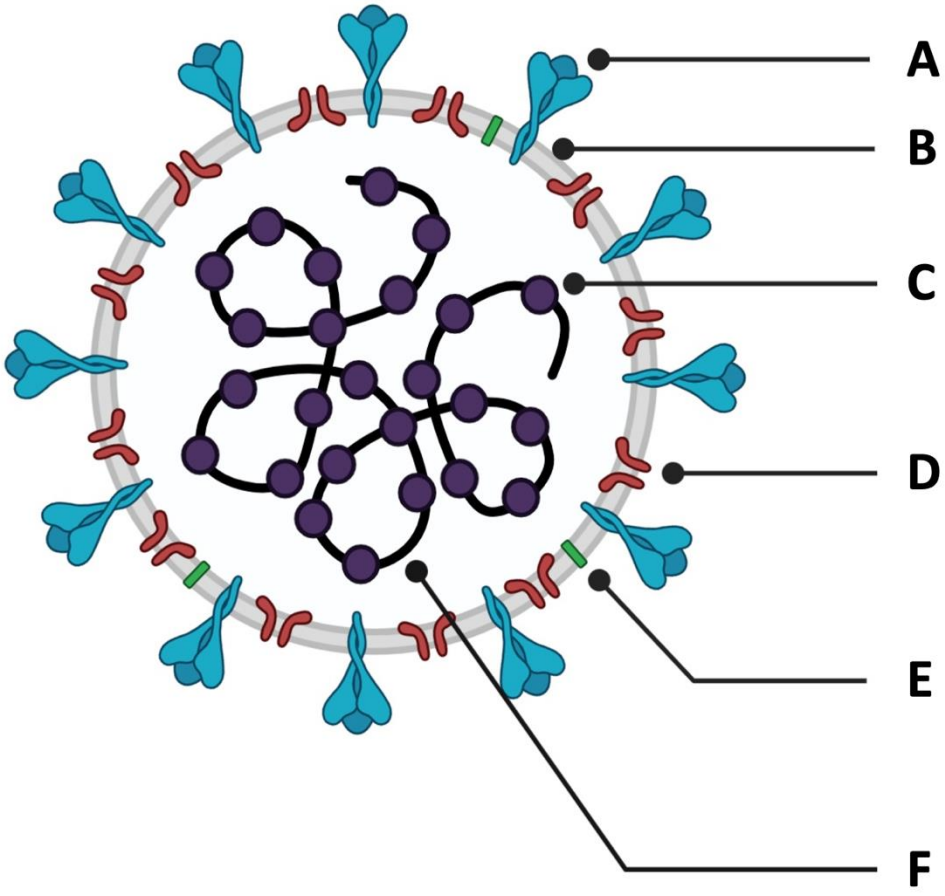
An animated story by the WHO explaining how SARS-CoV-2 spreads mainly between people in close contact with each other. It spreads most easily in crowded settings, closed spaces with poor ventilation or through prolonged contact with an infected person.

Link: https://www.youtube.com/watch?v=oqFn6AHoJZQ

Assessment Activity: What Have You Learned?

1. Explain how the designations COVID-19, SARS-CoV-2 and coronavirus mean different things, and why is it important to be precise when dealing with microbial nomenclature. Which coronavirus is the closest relative to SARS-CoV-2?

2. The diagram below represents a SARS-CoV-2 virus. Try to identify as many structures as you can and write one sentence describing the role of spike glycoprotein (S protein) in causing the disease.



3. In a hypothetical example, how many particles would be released if each of the 1,000 particles that originally entered the lungs continue to infect cells and create 1,000 more particles each in 10 additional hours (e.g., by 20 hours)? What happens in the next ten hours? Calculate and fill in the number or particles released at 30, 40 and 50 hours. On the bottom row, change the numbers to exponential notation.

Time from infection	10 hours	20 hours	30 hours	40 hours	50 hours
SARS-CoV-2 particles released	1,000	1,000,000			
Exponential	10^3				

4. Imagine you plan 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 tests explained above would you choose, or what other information would you want to learn before selecting the test? Use evidence from the table to support your answer.

5. Imagine that three different individuals get tested for COVID-19 with three different tests. Which results among those can be attributed to the current infection, and which one to past infection?

Individual	**RT-PCR test**	**Antigen test**	**Antibody test**
1	Positive	Positive	Negative
2	Positive	Negative	Negative

3	Negative	Negative	Positive

The Microbiology of SARS-CoV-2 – Science Major and Upper-Level College Students

Learning Outcomes:

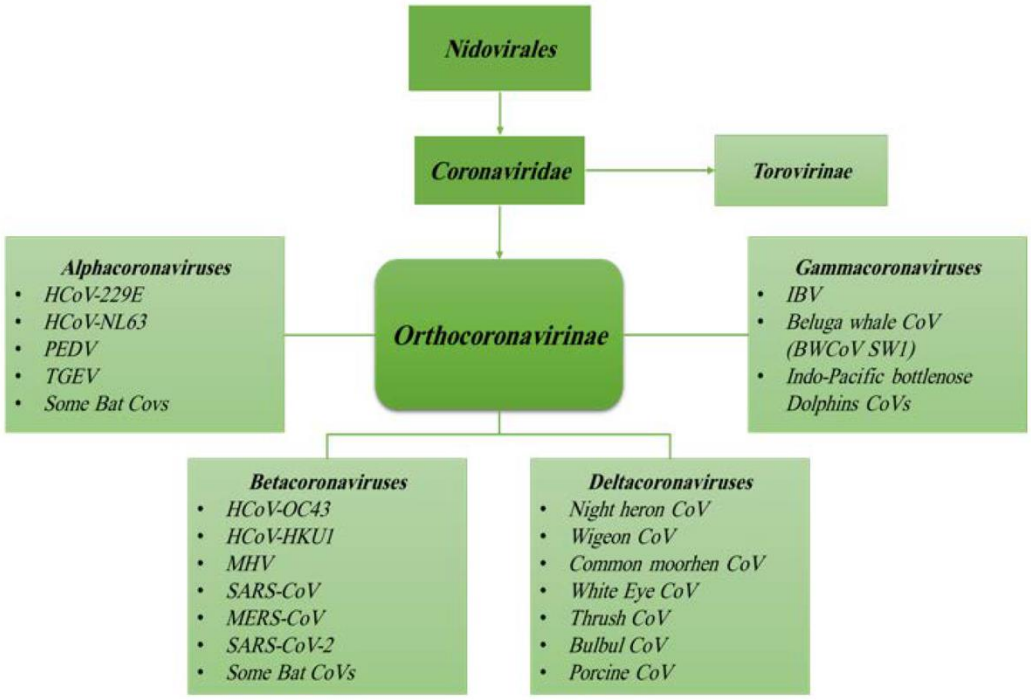
- Understand taxonomy/classification of coronaviruses within different viral classification systems and compare different coronaviruses
- Appraise virion structure of SARS-CoV-2 and other coronaviruses and put it into context of infecting a cell
- Comprehend the reasons for high SARS-CoV-2 infectivity and spread
- Explain physical and chemical resistance of SARS-CoV-2 and put it into context of enabling viral survival
- Compare and contrast different viral detection methods

Background Lesson and Reading: From Structure to Detection

Taxonomy: Coronavirus disease (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This virus belongs to the *Coronaviridae* family under the order *Nidovirales* (nido is a Latin word for "nest") – with highly conserved genomic organization and 3' nested subgenomic messenger RNAs (mRNAs). There are four subgroups of the coronavirus family: alpha (α), beta (β), gamma (γ), and delta (δ) coronavirus. The four "common human coronaviruses" are 229E (α coronavirus), NL63 (α coronavirus), OC43 (β coronavirus) and HKU1 (β coronavirus) (Figure 1). SARS-CoV-2 is actually a β-coronavirus, which is a group that also includes SARS-CoV and MERS-CoV – two other notable acute lung-injury causing coronaviruses of zoonotic origin (Figure 1). SARS-CoV-2 is most closely related to SARS-CoV, and shares roughly 80% identity at a nucleotide level. SARS-CoV-2 and other coronaviruses also belong to the fourth Baltimore group that contains viruses that have a positive sense single-stranded RNA (+ssRNA) genome. Remember, Baltimore classification is a system used to classify viruses based on their manner of mRNA synthesis. Do you know any other viruses that belong to this group?

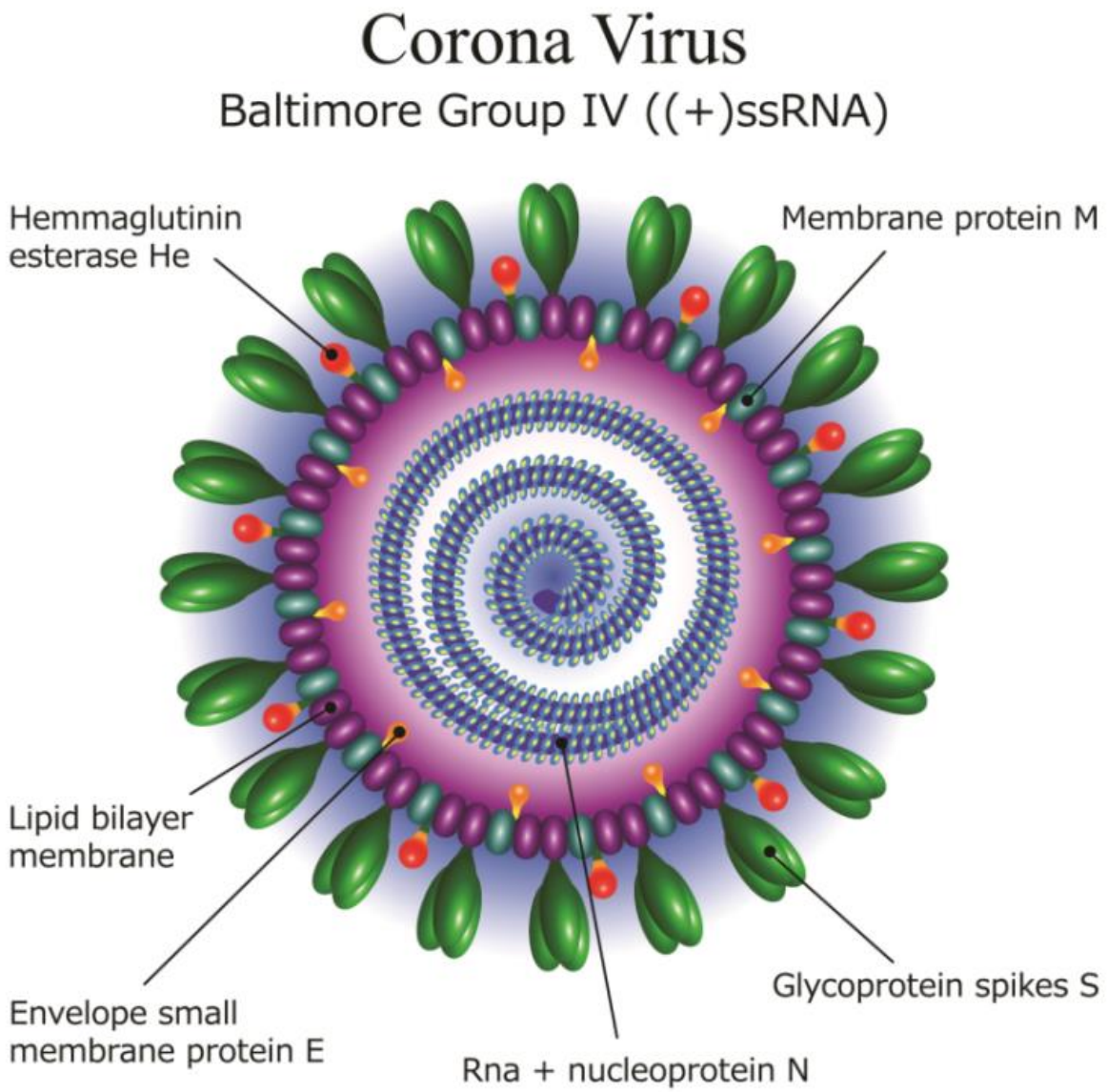
Structure: Coronaviruses are small (i.e., 65-120 nm in diameter) encapsulated viruses that have a characteristic crown-like appearance visible under an electron microscope, which is largely due to the presence of spike glycoproteins on the envelope. This is actually the reason behind the name for the whole viral family ('corona' in Latin translates as 'the crown'). Coronaviruses have large (26-32 kb) single-stranded, positive-sense RNA genomes. The genome is split into 14 open reading frames, which entails 16 nonstructural proteins and four structural proteins. Basically, coronaviruses come with four canonical structural proteins: the large transmembrane spike protein (S) with 1160–1400 amino acids, an integral and abundant membrane glycoprotein (M) with 250 amino acids, a small envelope protein (E) with 74–109 amino acids, and a heavily phosphorylated nucleocapsid protein (N) with 500 amino acids. There is also a hemagglutinin esterase (HE) protein that is not essential for viral replication *in vitro*, but may affect the production of infectious viral particles and viral tropism the human organism. Trimers of S protein are known to form 18–23 nm-long spikes on the surface of coronavirus, which give its characteristic aforementioned morphology. One outcome of this module is to get acquainted with the diagram of coronavirus particle structure (Figure 2), which is important to recognize in the era of pervasive pandemic infographics.

Figure 1: Coronaviruses with notable respiratory effects



(source: Hozhabri H et al. doi: 10.3390/ijerph17165648)

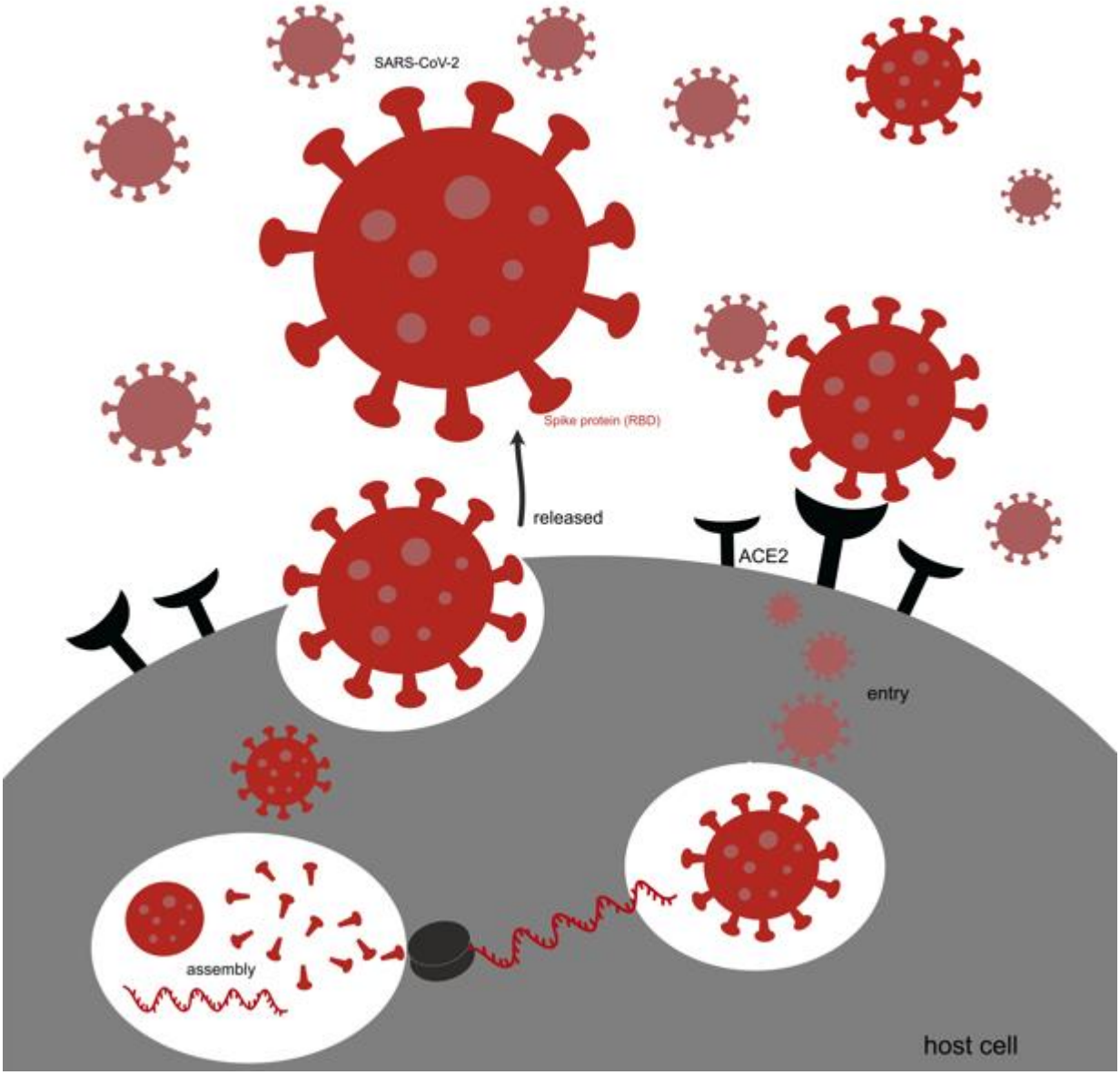
Figure 2: General structure of a coronavirus particle



Cell entry and viral replication: Why is SARS-CoV-2 so much more infectious than SARS-CoV or MERS-CoV? One possible reason may be in how this virus can gain access to host cells. Viral binding to the cells occurs via the interaction of the S protein of SARS-CoV-2 with Angiotensin-converting enzyme 2 (ACE2), which is a specific protein on the surface of the cell that acts like a receptor (primarily expressed on the surface of lung alveolar epithelial cells and enterocytes of the small intestine). ACE2 is also present in arterial/venous endothelial cells, and in arterial smooth muscle cells of multiple organs. After binding to the receptor, the virus must gain access to the host cell cytosol which, for coronaviruses, usually entails proteolytic cleavage of the S protein, followed by the fusion of the viral and cellular membranes. Data indicate that the priming of its S protein for membrane fusion involves the protease TMPRSS2, while the fusion process ultimately leads to the release of the viral RNA genome into the cell cytoplasm. The next steps leading to viral replication would involve viral RNA translation, assembly of viral replicase transcription complexes, viral RNA synthesis, assembly of virions, and transport of these to the cell surface inside vesicles, as explained in the Physiology module. The newly formed infectious virions are then released from the host cell by exocytosis. The summary of this infectious cycle can be seen on Figure 2.

Physical and chemical resistance: In the absence of any ventilation, SARS-CoV-2 remains viable in aerosols for 3 hours, with median half-life of 1.1-1.2 hours. The virions are most stable on stainless steel and plastic, with viable virus detected up to 72 hours (median half-life of 5.6 hours on steel and 6.8 hours on plastic) in the absence of surface disinfection. Importantly, on all surfaces and in the air, there is an exponential decay in virus titer over time. Furthermore, SARS-CoV-2 is more stable on smooth surfaces like glass, steel and plastic (several days) than on rough surfaces like paper, wood and cloth (several hours). Like other coronaviruses, SARS-CoV-2 is very stable at 4 °C but sensitive to ultraviolet rays and heat (regarding the latter, the virus is inactivated within 5 minutes at 70 °C). In addition, these viruses can be effectively inactivated by lipid solvents and common disinfectants – including ether (75%), chlorine-containing disinfectant, ethanol, chloroform and peroxyacetic acid. Soaps can dissolve the lipid bilayer of various viruses, thus they also induce SARS-CoV-2 inactivation.

Figure 3: Summary of the infectious cycle of SARS-CoV-2



Transmission: SARS-CoV-2 is transmitted from human to human by infectious droplets. More specifically, contact tracing studies show that prolonged close contact represents the main risk factor for transmission and that the risk of infection is much higher in household contacts compared to non-household contacts. Transmission may also occur indirectly via contaminated surfaces or fomites, even though that risk is considered to be low. SARS-CoV-2 viral RNA has not only been found in upper respiratory tract secretions, but also in other body fluids such as blood, feces and (in rare instances) urine. The potential of long-range airborne transmission of SARS-CoV-2 is also widely accepted, and the inhalation of very fine respiratory droplets and aerosol particles is also a way of viral transmission. Lung cells are the prime target of the virus, and this is the ideal point to consider the size difference between a SARS-CoV-2 virus particle and the cell it infects (which are basically type 1 alveolar epithelial cells located deep in the lung). Those lung cells are around 80 µm, so you could actually line up 800 viral particles across the diameter of a single lung cell. Furthermore, a single viral particle that infects a single lung cell can, following the exponential multiplication, release 1,000 replicated viral particles over the subsequent ten hours. Each of these particles can infect other lung cells, or be breathed out through the lung into the air in either droplets (after cough or sneeze) or microdroplets (even when just breathing). Do you remember the concept of exponential growth and what does it mean?

Viral detection: The so-called 'gold standard' for SARS-CoV-2 diagnostics are molecular methods, which primarily involve reverse transcriptase polymerase chain reaction (RT-PCR). These assays seek to qualitatively detect nucleic acid from SARS-CoV-2 on upper respiratory tract samples (such as nasopharyngeal or oropharyngeal specimens), but also lower respiratory tract samples (such as bronchoalveolar lavage). First line testing usually entails upper respiratory tract samples, which are much easier to take and come with lower viral transmission risk; on the other hand, lower respiratory tract samples are reserved for selected hospitalized cases with COVID-19. RT-PCR has also been used on other sample types, including blood or fecal samples, although these are not used for diagnostic work-up. Immunological assays, or serology tests, have been developed to detect/measure antibodies directed against SARS-CoV-2 proteins from blood. Currently available assays target the main immunogenic coronavirus proteins: the N-protein, the S-protein or the Receptor Binding Domain of the S-protein (RBD).

Rapid tests have been developed with the underlying idea of a point-of-care approach, offering rapid results (within 10-30 minutes). There are currently rapid tests for both the detection of antigens and the detection of antibodies. Rapid antigen tests are immune-chromatographic assays (lateral flow tests) which involve the detection of SARS-CoV-2 antigen in respiratory samples. Conversely, rapid antibody tests are immune-chromatographic assays developed for the detection of circulating SARS-CoV-2 antibodies from the blood. The tests provide rapid but only qualitative information (i.e., are IgM and/or IgG antibodies present or not). The table below summarizes and compares some aspects of these tests.

Table 1: Test methods for COVID-19

Type of test	**Testing for the presence of**	**Test accuracy**	**Time to obtain results**
RT-PCR test	Pieces of the viral RNA genome	Few false negatives (which means an active infection is not detected by the test). Test usually does not have to be repeated.	From one day to one week
Antigen test	Parts of viral proteins (also known as antigens)	More false negatives in comparison the RT-PCR test. May necessitate the use of other tests to confirm negative results.	One hour or less

Interactive Activity: Explore the SARS-CoV-2 Structure and Inner Workings

An interactive graphical story: Inside the Coronavirus

What scientists know about the inner workings of the pathogen that has infected the world by Scientific American.

Link: https://www.scientificamerican.com/interactive/inside-the-coronavirus/

Coronavirus Anatomy Explained: Science, Simplified

An animated look at the inner workings of the coronavirus that causes COVID-19. Illustrated by a Scripps Research scientist, this installment of Science, Simplified gives an overview of the key elements of SARS-CoV-2.

Link: https://www.youtube.com/watch?v=8hgc2iZflTI

World Health Organization (WHO): How COVID-19 is transmitted

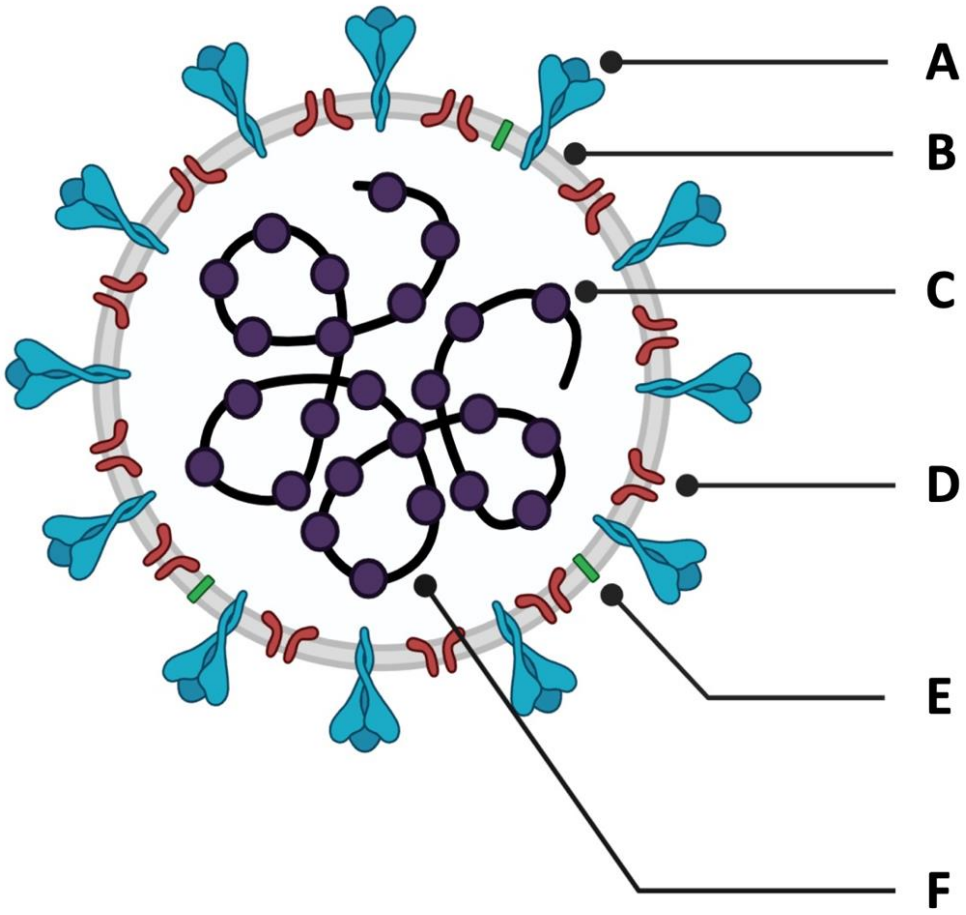
An animated story by WHO explaining how SARS-CoV-2 spreads mainly between people in close contact with each other. It spreads most easily in crowded settings, closed spaces with poor ventilation or through prolonged contact with an infected person.

Link: https://www.youtube.com/watch?v=oqFn6AHoJZQ

Assessment Activity: What Have You Learned?

1. Explain how the designations COVID-19, SARS-CoV-2 and coronavirus mean different things, and why is it important to be precise when dealing with microbial nomenclature. Which coronavirus is the closest relative to SARS-CoV-2?

2. The diagram below represents a SARS-CoV-2 virus. Identify each structure and write one sentence describing its role in viral structure and/or pathogenesis.



3. In a hypothetical example, how many particles would be released if each of the 1,000 particles that originally entered the lungs continue to infect cells and create 1,000 more particles each in 10 additional hours (e.g., by 20 hours)? What happens in the next ten hours? Calculate and fill in the number or particles released at 30, 40 and 50 hours. On the bottom row, change the numbers to exponential notation.

Time from infection	10 hours	20 hours	30 hours	40 hours	50 hours
SARS-CoV-2 particles released	1,000	1,000,000			
Exponential	10^3				

4. Imagine you plan 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 tests explained above would you choose, or what other information would you want to learn before selecting the test? Use evidence from the table to support your answer.

5. Imagine that three different individuals get tested for COVID-19 with three different tests. Propose a possible explanation for each individual's test results, as depicted in the following table.

Individual	**RT-PCR test**	**Antigen test**	**Antibody test**	**Explanation**
1	Positive	Positive	Negative	
2	Positive	Negative	Negative	

3	Negative	Negative	Positive	

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What Is Coronavirus?: Coronavirus: How Does It Work? 2020 Baylor College of Medicine

The Genetics of SARS-CoV-2: Variants – High School, Informal Learners, and Non-Science Major College Freshman

Learning Outcomes:

- Be able to explain what a variant is, and the difference between a variant and a mutation.
- Learn how interpret sequence information.
- Be able to recognize and describe mutations in a sequence.

Background Lesson and Reading: What is a genetic variant?

The SARS-CoV-2 genome: SARS-CoV-2 is an RNA virus, meaning that its genome is made of RNA. Specifically, it is a single strand of RNA that is approximately 30 kilobases (30,000 bases), which is fairly large for an RNA genome. It has at either end a 5'-cap and 3' poly(A) tail, allowing it to act as messenger RNA (mRNA) to be translated by machinery within the host cell (Romano et al., 2020). This means that a cell infected with SARS-CoV-2 can use the viral genome as direct instructions for making proteins. The SARS-CoV-2 genome encodes for about 50 different proteins, some of which make up the structure of the virus, but many of which have other functions in the biology of the virus; a diagram of the full genome is shown in Figure 1, with different genes shown as rectangles along its length. Genes encoding structural proteins are shown in red, including the spike protein (S), small envelope protein (E), membrane glycoprotein (M), and nucleocapsid protein (N).

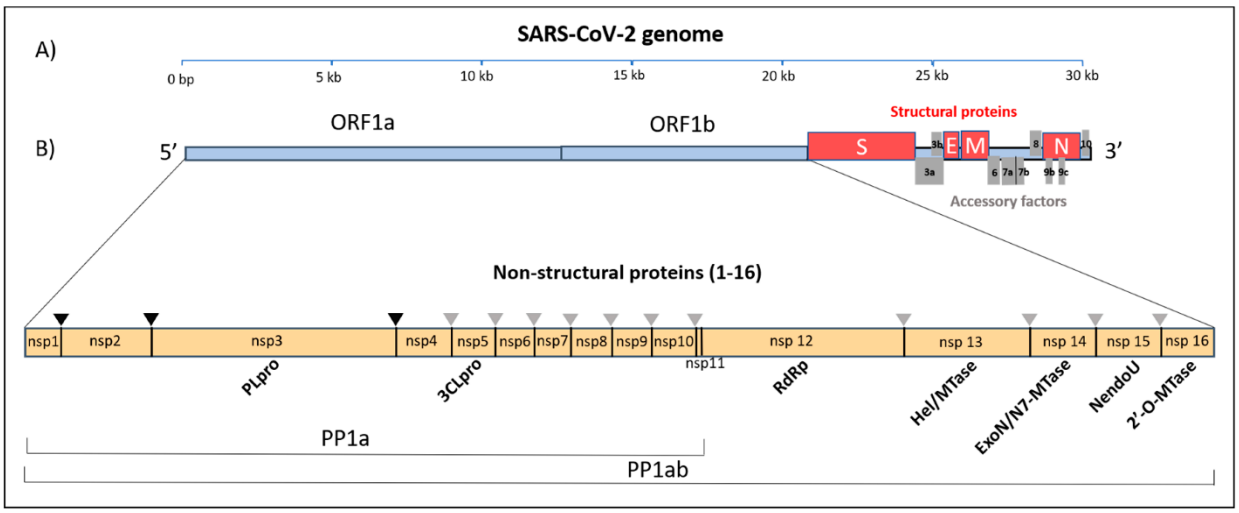


Figure 1: SARS-CoV-2 19 genome (Romano et al., 2020).

Learning about variants: Go to this site from the Global Virus Network and answer the questions below: https://gvn.org/ Useful information on mutations and variants can also be found here on this site from the CDC: https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html

1. Explain the terms mutation and variant in your own words. What is the relationship between these two?
2. Look through the GVN page to find information on the Delta variant. Look for a list of mutations that define this variant; these are positions in a protein where due to mutations to the genome an amino acid was changed. These aren't necessarily all of the changes to this variant, but those considered most important in defining the variant. (Hint: look in the blue box on the left on the Delta Variant page)
    1. In which protein are the listed mutations found?
    2. How many mutations are listed?

Interactive Activity: Explore the SARS-CoV-2 genome

We will compare the sequence of the spike gene of SARS-CoV-2 from the first identified human patient to the sequence of the Delta variant. Figure 2 shows the

first strain on the top, and from the Delta variant on the bottom. The sequence made up of a, t, g, and c shows the sequence of the genome; the first 60 nucleotides are shown (the numbers are listed below the sequence). The letters in colored blocks beginning from the left with "M, F, V, F…" show the amino acids coded by the genome sequence; the codon for each amino acid is a 3-nucleotide sequence. The amino acids show the sequence of the protein that will be made. This figure shows the first 60 nucleotides of the gene (positions 1 to 60), which corresponds to the first 20 amino acids of the spike protein.

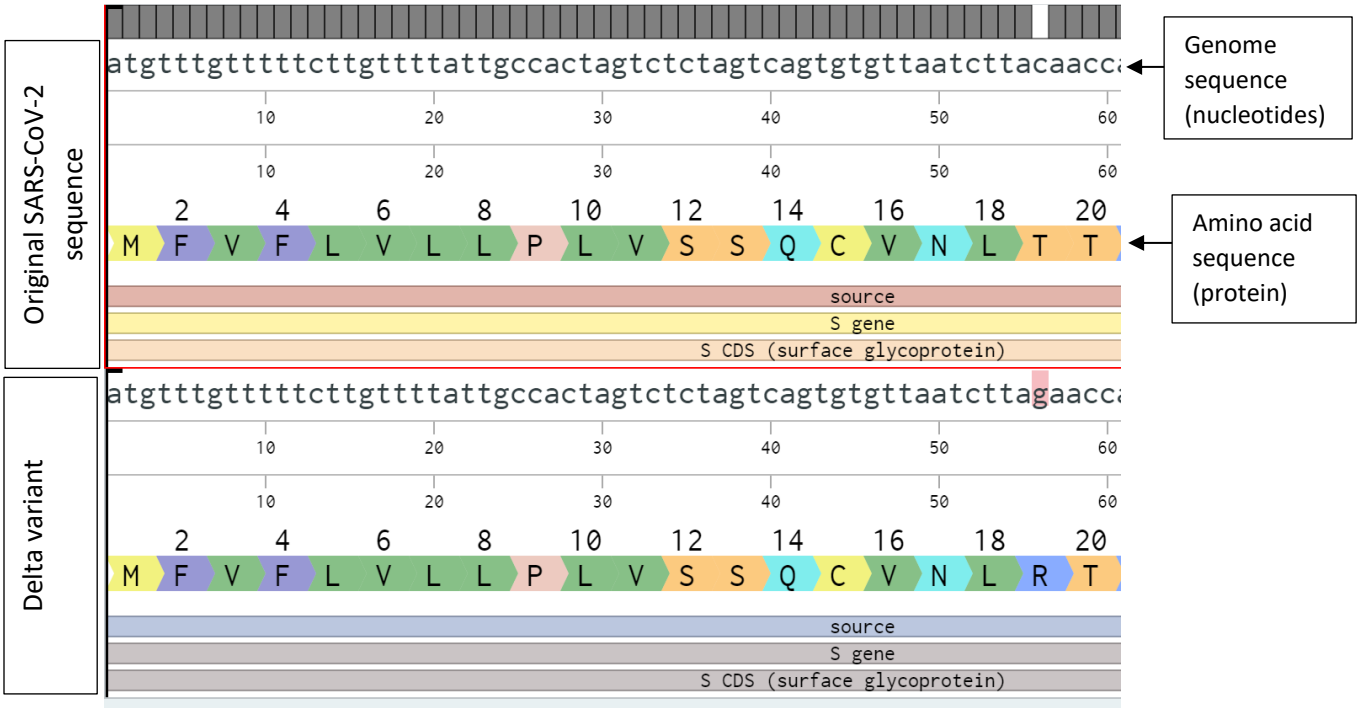
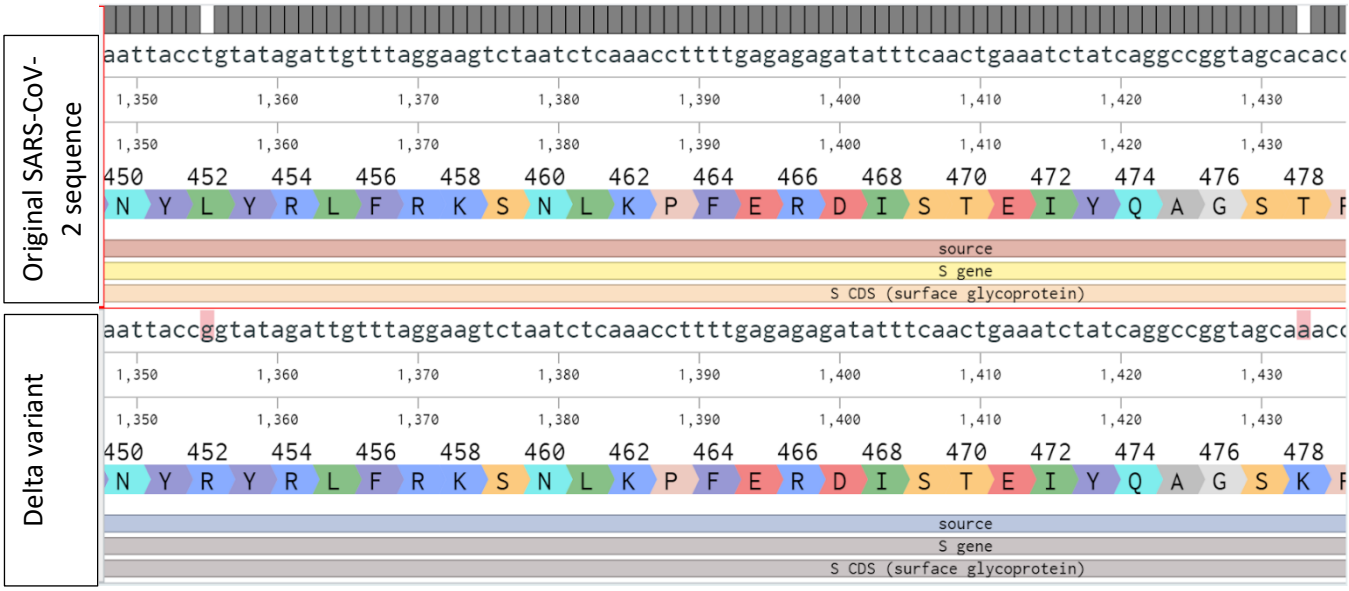


Figure 2: Aligned sequences of the SARS-CoV-2 19 genome from the first infected human patient (top) and the sequence of the Delta variant (bottom). Images generated using Benchling.com.

These two sequences are aligned to identify differences more easily. Look at position 56 in the genome sequence; in the original sequence this is a "c" (cytosine) but in the Delta variant it is a "g" (guanine). Because of this change, the amino acid at position 19 was "T" (Threonine) in the original spike protein but was changed to "R" (Arginine) in the Delta variant. Go back to the list of substitutions in the Delta variant listed on the CDC website; the first spike protein substitution listed, "T19R", describes the change you can see in the aligned sequence in Figure 2.

Assessment Activity: What Have You Learned?

Practice identifying more mutations in the Delta variant spike protein. Use the alignment shown below to answer the following questions.



1. Which part of the spike protein genome is shown here – which nucleotides? Which amino acids? In other words, what is the number range of each?

2. How many mutations are present in this part of the genome?

3. For each mutation, describe the change in the genome and the change in the amino acid – which position, what is it in the original, and what is it in the Delta variant?

The Genetics of SARS-CoV-2: Variants – Non-Science Major and Lower-Level College Students

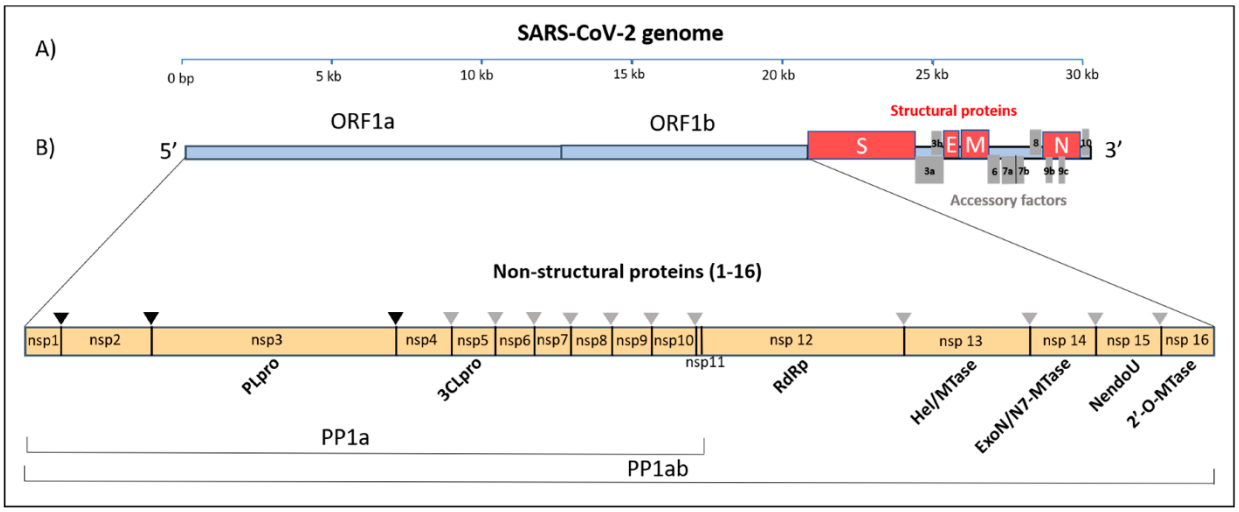
Learning Outcomes:

- Be able to explain what a variant is, and the difference between a variant and a mutation.
- Learn how to retrieve and view sequence information
- Identify features in sequence information
- Relate changes in the genome to the idea of a genetic variant

Background Lesson and Reading: What is a genetic variant?

The SARS-CoV-2 genome: SARS-CoV-2 is an RNA virus, meaning that its genome is made of RNA. Specifically, it is a single strand of RNA that is approximately 30 kilobases (30,000 bases), which is fairly large for an RNA genome. It has at either end a 5'-cap and 3' poly(A) tail, allowing it to act as messenger RNA (mRNA) to be translated by machinery within the host cell (Romano et al., 2020) The SARS-CoV-2 genome encodes for about 50 different proteins, some of which make up the structure of the virus, but many of which have other functions in the biology of the virus; a diagram of the full genome is shown in Figure 1. Genes encoding structural proteins are shown in red, including the spike protein (S), small envelope protein (E), membrane glycoprotein (M), and nucleocapsid protein (N).

Figure 1: SARS-CoV-2 19s genome (Romano et al. 2020)



Learning about variants: Look through the following pages from the CDC (https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html) and the Global Virus Network (https://gvn.org/) and answer the questions below.

1. Explain mutation, lineage, and variant in your own words. What is the relationship between the terms mutation and variant?
2. Look through the GVN page to find information on the Delta variant. Look for a list of mutations that define this variant; these are positions in a protein where due to mutations to the genome an amino acid was changed. These aren't necessarily all of the changes to this variant, but those considered most important in defining the variant. (Hint: look in the blue box on the left on the Delta Variant page)
    1. In which protein are the listed mutations found?
    2. How many mutations are listed?

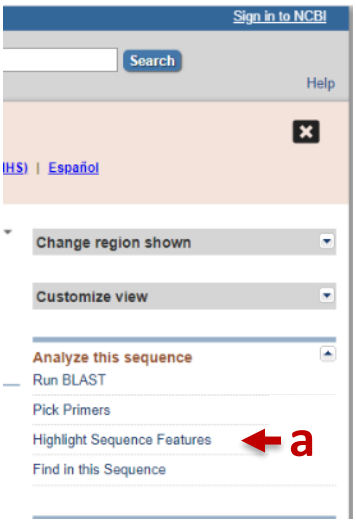
Interactive Activity: Explore the SARS-CoV-2 genome

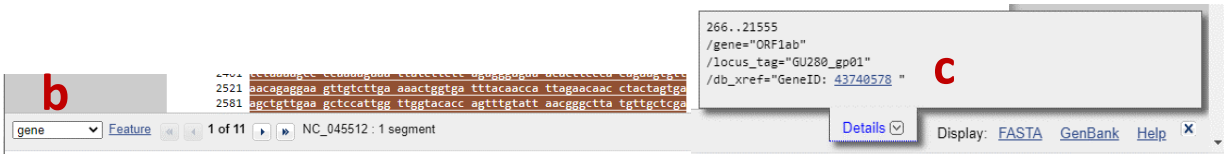
We will use the National Center for Biotechnology Information (NCBI) database to locate the sequence for the full SARS-CoV-2 human genome, from patient zero in Wuhan, China.

1. Go to https://www.ncbi.nlm.nih.gov/ to open the NCBI site.
2. The dropdown menu at the top of the page should read “All Databases”; keep this setting. Enter the accession number for the Wuhan Human coronavirus into the search bar: NC_045512.2
3. This should pull up a listing for a nucleotide sequence with the title: “Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome”. Click on the link under the name to bring you to a page with the nucleotide information – the sequence, along with detailed annotations.
    a. Record: Exactly how many base pairs long is this nucleotide sequence?

    b. Record: What is the most recent journal reference cited for this sequence?

4. Find the part of the sequence that encodes the spike protein:
    a. Click on “Highlight sequence features”. This is on the column on the right under “Analyze this sequence”.
    b. On the lower left of the screen, there is a dropdown menu – change it to “gene”.
    c. On the right side of the screen along the bottom, be sure that the Details are showing – click the arrow to show/hide the details.
    d. Use the arrows on the bottom bar in the browser window to scroll through genes until the information under details shows the spike protein (gene = “S”)





    e. Record: What are the base pairs that code for the spike protein? How many base pairs long is the gene that encodes the spike protein?

f. Record: What is the GeneID for the spike protein? Note that this number can be used to search for sequence information on the protein itself.
g. Change the dropdown menu to CDS, which indicates sequences that code for proteins. Scroll until once again fine the spike protein, gene = S. The details window should now show the translation of this gene, meaning the protein sequence with each amino acid designated by its single letter code.

Remember, you are viewing the sequence of the SARS-CoV-2 human genome from patient zero. A variant has mutations in the genomic sequence that lead to amino acid substitutions that lead to changes in the protein. These mutations happen naturally during viral replication, and in general those that confer some competitive advantage will become more frequent (see "Genetic Variants of SARS-CoV-2 – What Do They Mean?" by Adam Lauring and Emma Hodcroft. https://jamanetwork.com/journals/jama/fullarticle/2775006) if you would like more information.

Assessment Activity: What Have You Learned?

Look over this site discussing the mutations in omicron variants: https://www.washingtonpost.com/health/2021/12/16/omicron-variant-mutations-covid/

Choose one mutation in the spike protein of the omicron variant. Clearly describe what amino acid position is changed and what it is changed to, then discuss what advantage this change gives the virus.

The Genetics of SARS-CoV-2: Variants – Science Major and Upper-Level College Students

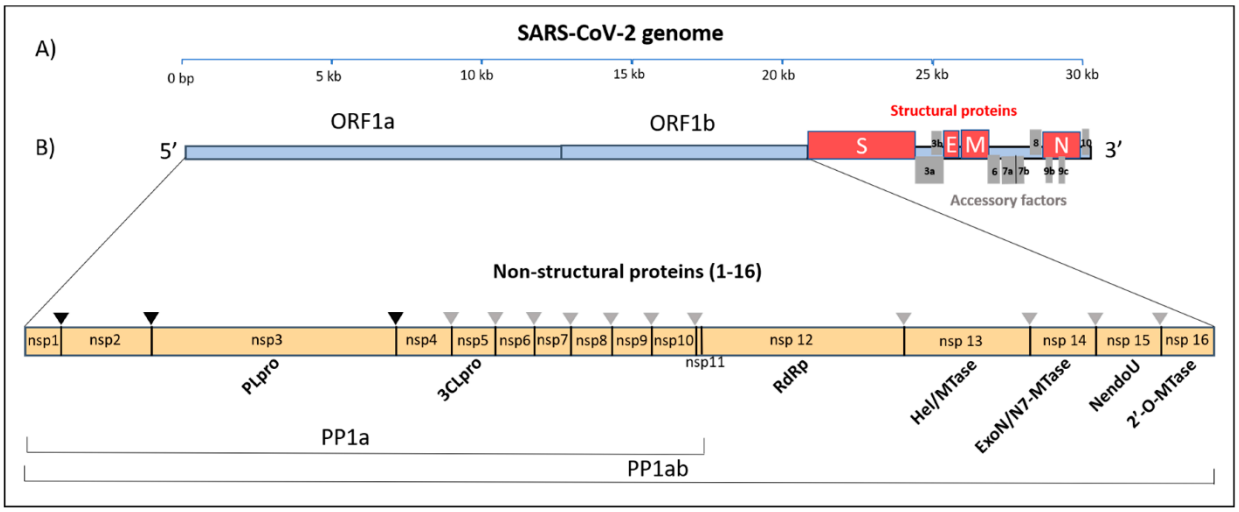
Learning Outcomes:

- Be able to explain what a variant is, and the difference between a variant and a mutation.
- Learn how to retrieve and view sequence information
- Identify features in sequence information
- Align sequences to make comparisons
- Relate changes in the genome to the idea of a genetic variant

Background Lesson and Reading: What is a genetic variant?

The SARS-CoV-2 genome: SARS-CoV-2 is an RNA virus, meaning that its genome is made of RNA. Specifically, it is a single strand of RNA that is approximately 30 kilobases (30,000 bases), which is fairly large for an RNA genome. It has at either end a 5'-cap and 3' poly(A) tail, allowing it to act as messenger RNA (mRNA) to be translated by machinery within the host cell (Romano et al., 2020) The SARS-CoV-2 genome encodes for about 50 different proteins, some of which make up the structure of the virus, but many of which have other functions in the biology of the virus; a diagram of the full genome is shown in Figure 1. Genes encoding structural proteins are shown in red, including the spike protein (S), small envelope protein (E), membrane glycoprotein (M), and nucleocapsid protein (N).

Figure 1: SARS-CoV-2 19s genome (Romano et al. 2020)



Learning about variants: Read this short article about SARS-CoV-2 variants: "Genetic Variants of SARS-CoV-2 – What Do They Mean?" by Adam Lauring and Emma Hodcroft. https://jamanetwork.com/journals/jama/fullarticle/2775006

Next, Look through the following pages from the CDC (https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html) and the Global Virus Network (https://gvn.org/).

Answer the questions below:

1. What is the relationship between the terms mutation, variant, and strain?
2. Choose one substitution in the Delta variant (B.1.617.2), as listed on the GVN website, and explain what mutation could lead to that change.
    a. State the specific substitution you are discussing.
    b. What amino acid was in the position originally, and what is/are the RNA codons for that amino acid?
    c. What is the new amino acid in that position, and what is/are the RNA codons for that amino acid?
    d. Give an example of a mutation that could have resulted in the amino acid substitution.

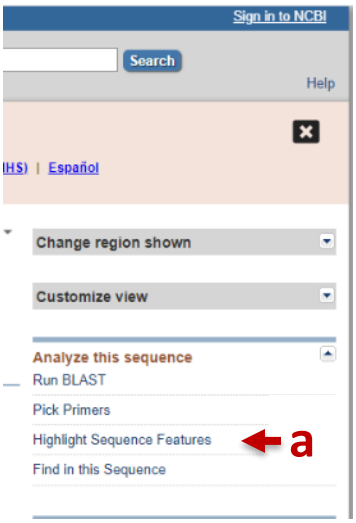
Interactive Activity: Explore SARS-CoV-22 Genome Variants

We will first use the National Center for Biotechnology Information (NCBI) database to locate the sequence for the full SARS-CoV-2 human genome, from patient zero in Wuhan, China. Then we will compare this spike protein sequence with the sequence from a SARS-CoV-2 Variant.

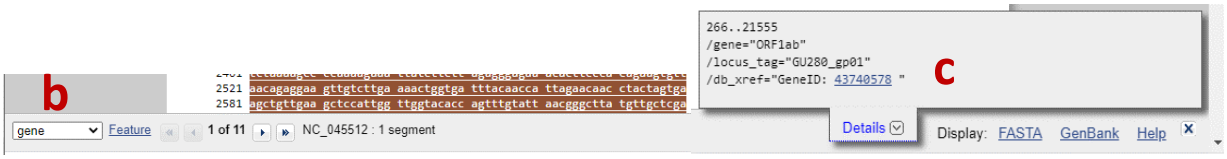
1. Go to https://www.ncbi.nlm.nih.gov/ to open the NCBI site.
2. The dropdown menu at the top of the page should read "All Databases"; keep this setting. Enter the accession number for the Wuhan Human coronavirus into the search bar: NC_045512.2
3. This should pull up a listing for a nucleotide sequence with the title: "Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome". Click on the link under the name to bring you to a page with the nucleotide information – the sequence, along with detailed annotations.
    a. Record: Exactly how many base pairs long is this nucleotide sequence?

    b. Record: What is the most recent journal reference cited for this sequence?

4. Find the part of the sequence that encodes the spike protein:
    a. Click on "Highlight sequence features". This is on the column on the right under "Analyze this sequence".
    b. On the lower left of the screen, there is a dropdown menu – change it to "gene".
    c. On the right side of the screen along the bottom, be sure that the Details are showing – click the arrow to show/hide the details.



d. Use the arrows on the bottom bar in the browser window to scroll through genes until the information under details shows the spike protein (gene = “S”)



e. Record: What are the base pairs that code for the spike protein? How many base pairs long is the gene that encodes the spike protein?
f. Record: What is the GeneID for the spike protein? Note that this number can be used to search for sequence information on the protein itself.

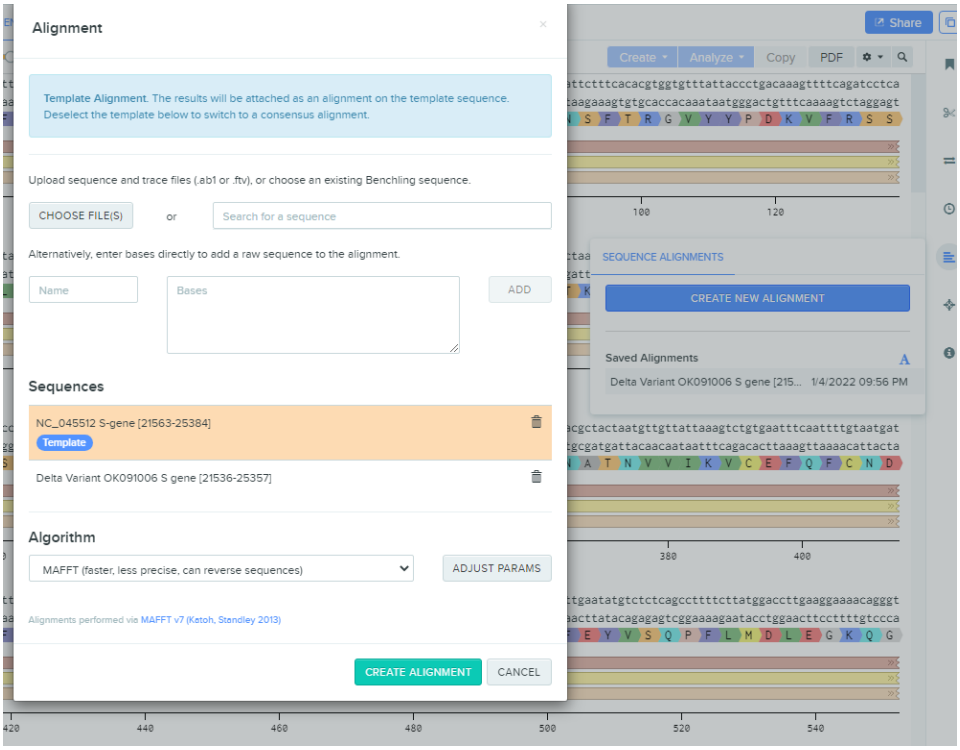
Next, you will use a sequence analysis platform to view features of the SARS-CoV-2 sequence, extract the sequence of just the spike protein itself, and align it to the sequence of the Delta variant to look for differences.

To complete this next part of the activity, create an account with Benchling. Academic accounts are free and can be set up here: https://benchling.com/signup/welcome.

Once you have an account, watch this video tutorial to orient yourself to the Benchling Platform: https://youtu.be/KOCM-tpbe5o. This Benchling video tutorial may also be helpful as you work through this exercise: Introduction to Benchling Molecular Biology: https://youtu.be/_C6-oSbpvWM

- Set up a new project in Benchling and import your sequence:
  - Create new project
  - Import sequence: From the project tab on the left, click “+”, DNA Sequence -> New DNA sequence -> Search External Databases -> Import (search for the accession number NC_045512.2)
- Quick tutorial of the layout/features:
  - Explore the workspace: Along the top, look at the Sequence Map, Linear Map, Description. On the lower right, toggle “Split Workspace”.

    - Within the sequence map, highlight a portion of the sequence. Note that along the bottom of the window it shows the starting and ending base number and the length of the highlighted region.
    - On the right side of the window, find the symbol that brings up the list of annotations, click on it to see the list.
- This is the entire genome of this particular SARS-CoV2-Variant; next, find just the gene for the spike protein and copy it to a new file.
    - Click on "S gene" to highlight; this is easiest in the linear map view.
    - Right click on the highlighted gene to bring up the options menu. Select "Create DNA Sequence". Save in your current Benchling project.
    - This creates a new file with just the sequence of the spike gene from patient zero.
    - Highlight the entire sequence of the spike gene. In the upper right select "Create -> Translation -> Forward" to see the amino acid sequence alongside the DNA sequence.
- Repeat the same steps to import the sequence of the Delta Variant (accession number OK091006), copy the sequence of the spike gene to a new file, and add the translation.
- Align the two spike protein sequences:
    - Go to the tab with the sequence of the patient zero spike protein. Click on the Alignments symbol on the bar on the right of the window. Select "Create New Alignment"
    - The sequence for your patient zero spike protein should already be listed as the Template Sequence. Start typing the name of your Delta variant sequence into the "Search for a sequence" bar and select it when found.



- You should now see both sequences listed under “Sequences”; leave the default algorithm selected (MAFFT), and click “Create Alignment”.
- Explore the alignment between the two sequences.
    - At the bottom of the window, the entirety of both sequences is shown, with differences highlighted as colored bars. Drag the selected region to explore along the sequence in more detail.
    - Differences are also highlighted at the top, where grey bars show “votes” for each base if the sequence matches the template.
    - A selection can be exported as a PDF file by highlighting a portion (click and drag on the sequence) and selecting “Export -> PDF - screen capture (selection)” in the upper right.

Assessment Activity: What Have You Learned?

Consider the Delta variant substitution you selected while completing question 2 of the background section. Find it in your aligned sequences.

1. State the specific substitution you are discussing. What is the actual amino acid change in the sequence that led to this particular amino acid substitution?
2. Highlight approximately 50-100 base pairs of sequence surrounding this substitution and export this region of the alignment as a PDF. Give this a figure title; be sure the identity of each sequence and the sequence and amino acid change are clearly labeled.
3. How does knowing a genetic mutation characteristic of a specific variant allow you to test a COVID-positive sample for the presence of that variant? Explain how to test for a specific variant in a COVID-positive sample. Use this site from ThermoFisher Scientific for reference: https://www.thermofisher.com/us/en/home/clinical/clinical-genomics/pathogen-detection-solutions/real-time-pcr-research-solutions-sars-cov-2/mutation-panel.html

References:

Berkowitz, B, Steckelberg, A. "Understanding omicron's many mutations". The Washington Post. Dec. 16, 2021. https://www.washingtonpost.com/health/2021/12/16/omicron-variant-mutations-covid/

Lauring AS, Hodcroft EB. Genetic Variants of SARS-CoV-2—What Do They Mean? JAMA. 2021;325(6):529–531. doi:10.1001/jama.2020.27124

Romano M, Ruggiero A, Squeglia F, Maga G, Berisio R. A Structural View of SARS-CoV-2 RNA Replication Machinery: RNA Synthesis, Proofreading and Final Capping. Cells. 2020; 9(5):1267. https://doi.org/10.3390/cells9051267

"SARS-CoV-2 Variant Classifications and Definitions". Content Source: National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases. Updated Oct. 4, 2021. https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html

The Genetics of SARS-CoV-2: Origins – High School, Informal Learners, and Non-Science Major College Freshman

Learning Outcomes:

- Understand why sister species to SARS-CoV-2 are close but not the same as this virus
- Understand how to use a percent similarity matrix to draw conclusions about the relatedness of virus species

Background Lesson and Reading: The Coronavirus family

What can the genome tell us about the origin of the SARS-CoV-2 virus? COVID-19 is caused by the SARS-CoV-2 virus, which is a member of the *Coronaviridae* family. This family of viruses, which cause respiratory diseases in humans, include many different strains including HKU1, SARS-CoV, and MERS-CoV. These viruses are also known to spread to humans from animals including bats, cats, and camels; for example, civet cats passed SARS-CoV to humans and camels passed MERS-CoV to humans. This is known as zoonotic transmission.

Read over this link to learn more about different members of the Coronavirus family: https://www.intechopen.com/chapters/77392

As viruses spread through humans and animals their nucleic acid undergoes mutations and other changes, leading over time to new variants and new viral strains. You can draw conclusions about the relatedness of two viruses based on the similarity of the nucleotide sequences constituting all of their genes, or their genomes. Strains that are more closely related will share similar nucleotide sequences, while strains that diverged a longer time ago will have had more time to undergo changes and will have nucleotide sequences that are less similar. Therefore comparing the genome of SARS-CoV-2 virus to other related viruses can give us one piece of information about its possible origin.

Interactive Activity: Comparing Genomes

Table 1 was generated by comparing what percent of the genome – the DNA sequence – is the same between different viruses in the coronavirus family. The same set of coronavirus family members are listed left to right and top to bottom in the table. When a virus is compared against itself, the table shows 100%; the genome is exactly the same. Note which squares on the table show 100%. Now, use the table to compare the similarity of the genome between HKU1 (Human CoV) and SARS-CoV. What percent of the genome is the same between these two viruses? Which two squares in the table show you this same information? This comparison table is known as a percent similarity matrix.

Table 1: The percent similarity between the genomes of three viruses in the coronavirus family

	HKU1 Human CoV	**MERS-CoV**	**SARS-CoV**
HKU1	100.00%	52.75%	52.12%
MERS-CoV	52.75%	100.00%	53.19%
SARS-CoV	52.12%	53.91%	100.00%

Once you understand how to interpret Table 1, move on to Table 2. The first three viruses listed are the same, but this table adds SARS-CoV-2 (original human sequence), the omicron variant of SARS-CoV-2, and a bat coronavirus, Bat-CoV RaTG13.

Table 2: The percent similarity between the genomes of SARS-CoV2 and other viruses in the coronavirus family

	HKU1 Human CoV	**MERS-CoV**	**SARS-CoV**	**SARS-CoV-2**	**Omicron Variant of SARS-CoV-2**	**Bat-CoV RaTG13**
HKU1	100.00%	52.75%	52.12%	53.41%	53.43%	53.16%
MERS-CoV	52.75%	100.00%	53.19%	54.22%	54.19%	54.08%
SARS-CoV	52.12%	53.91%	100.00%	79.81%	79.60%	79.59%

SARS-CoV 2	53.41%	54.22%	79.81%	100.00%	99.70%	96.18%
Omicron Variant of SARS-CoV-2	53.43%	54.19%	79.60%	99.70%	100.00%	95.93%
Bat-CoV RaTG13	53.16%	54.08%	79.59%	96.18%	95.93%	100.00%

Notice how similar SARS-CoV-2 and the omicron variant of SARS-CoV-2 are to each other. This indicates that the genomes are different, but only by a small number of nucleotides. What percent of the genome of these two variants is the same? What percent is different?

Assessment Activity: What Have You Learned?

Use Table 2 to answer the following questions:

1. What virus has the most similar genome to SARS-CoV-2 and the omicron variant of SARS-CoV-2, and what percent of the genome is the same?
2. Based on Table 2, is SARS-CoV-2 more closely related to the other human coronaviruses (HKU1, MERS-CoV, SARS-CoV) or the bat coronavirus Bat-CoV?
3. What do these data from Table 2 suggest about the origin of SARS-CoV-2? Based on these data, is it more likely that SARS-CoV-2 came from animals or from other human coronaviruses? Explain. (Note that this is only one piece of evidence, and does not definitively prove the origin of the virus)

The Genetics of SARS-CoV-2: Origins – Non-Science Major and Lower-Level College Students

Learning Outcomes:

- Understand why sister species to SARS-CoV-2 are close but not the same as this virus
- Understand how to use a percent similarity matrix to draw conclusions about the relatedness of virus species

Background Lesson and Reading: The Coronavirus family

What can the genome tell us about the origin of the SARS-CoV-2 virus? COVID-19 is caused by the SARS-CoV-2 virus, which is a member of the *Coronaviridae* family. This family of viruses, which cause respiratory diseases in humans, include many different strains including HKU1, SARS-CoV, and MERS-CoV. These viruses are also known to spread to humans from animals including bats, cats, and camels; for example, civet cats passed SARS-CoV to humans and camels passed MERS-CoV to humans. This is known as zoonotic transmission.

Read over this link to learn more about different members of the Coronavirus family: https://www.intechopen.com/chapters/77392

Interactive Activity

Using the background reading and the following document, fill in Table 1 to compare the characteristics of different viruses.
https://link.springer.com/content/pdf/10.1007/978-3-7091-9163-7_8.pdf

Table 1: Virus characteristics. Indicate if the species has (Y) or does not have (N) the characteristics in the table.

Characteristics:	MERS-Cov	SARS-Cov	SARS-CoV 2	HKU1 Human CoV	Birnavirus IPNV
Enveloped Virus					
Contains S, M, and E membrane-associated proteins					
Contains a single nucleocapsid protein					
Single stranded RNA genome					
Infects upper respiratory tract mild to severe					

1. Based on these results, which of these species is NOT part of the coronavirus family? Support your decision.
2. Would a hypothesis stating "if you use the characteristics in Table 1, then you can distinguish the coronavirus species from each other" be supported or refuted? Explain your decision.

All of the characteristics in Table 1 are the products of transcription and translation. These processes read the codons from the nucleotide sequence of the virus genome. Ultimately polypeptides are produced that are directly or indirectly the components of the virus particle. Table 2 shows the similarity of the genomes as compared between different members of the coronavirus family. When a row and a column intersect, that cell represents the percent similarity between the virus genome in that row and the virus genome in that column. This is known as a percent similarity matrix. Notice that the same viruses are listed from left to right and from top to bottom, so that all comparisons are included; where a virus is compared against itself, the nucleotide sequence is identical, so it shows 100% similarity.

Table 2: The percent similarity matrix between the genomes of viruses in the coronavirus family

	HKU1 Human CoV	**MERS-CoV**	**SARS-CoV**	**SARS-CoV 2**	**Omicron Variant of SARS-CoV-2**	**Bat-CoV RaTG13**
HKU1	100.00%	52.75%	52.12%	53.41%	53.43%	53.16%
MERS-CoV	52.75%	100.00%	53.19%	54.22%	54.19%	54.08%
SARS-CoV	52.12%	53.91%	100.00%	79.81%	79.60%	79.59%
SARS-CoV 2	53.41%	54.22%	79.81%	100.00%	99.70%	96.18%
Omicron Variant of SARS-CoV-2	53.43%	54.19%	79.60%	99.70%	100.00%	95.93%
Bat-CoV RaTG13	53.16%	54.08%	79.59%	96.18%	95.93%	100.00%

3. Would a hypothesis stating "if you use the genome similarities in Table 2, then you can distinguish the coronavirus species from each other" be supported or refuted? Explain your decision.

Assessment Activity: What Have You Learned?

Answer the following questions:

1. One line of thought about the origin of SARS-CoV-2 is that it originated in one or more animal hosts (bat and/or pangolin are primary suspects) then jumped to humans. Are there any data in Table 2 that supports this line of thought as opposed to SARS-CoV-2 originating from MERS-CoV or SARS-CoV?
2. Genetic variability in sexually reproducing organisms occurs in a number of ways. Random fertilization within the population; during production of gametes (sperm and egg), independent assortment of the sets of chromosomes previously inherited is a big contributor. Switching sections

between chromosome pairs of the sets during meiosis is known as genetic recombination and also contributes to variation. Mutations along the nucleotide sequence of the genome are the original source of variation and occur in all organisms. How do you think coronavirus particles (the viruses) obtain variation? Support your answer.

3. Remember that viruses must use the transcription and/or translation process of the host cell to reproduce the viral genome and its other components. Within the family *Hominidae*, which includes humans and the other great apes, the percent similarities among species' genomes is 95% or higher. Why do you think the genome similarities among the viruses of the coronavirus family are so much lower?

The Genetics of SARS-CoV-2: Origins – Science Major and Upper-Level College Students

Learning Outcomes:

- Understand the terms phylogenetics, genome, and phylogenomics.
- Understand how these apply when filling in a phylogenetic tree/cladogram depicting the origin of SARS-CoV-2.

Background Lesson and Reading: Comparing genomes.

Phylogenetic trees and cladograms:

Phylogenetic trees and cladograms are prepared with the purpose of showing evolutionary relationships among species being studied. The species that are closer together in the process of divergence into different species should have similar characteristics compared to species that diverged from the lineage at a much earlier or later time. Additionally, divergence of organisms within the lineage is marked by the occurrence of particular traits within the divergent group that makes them different from other species in the ancestral lineage. These derived traits will continue to occur through mutation, natural selection and possibly other microevolution processes while maintaining many earlier shared traits. This exemplifies the definition of evolution as 'descent with modification'.

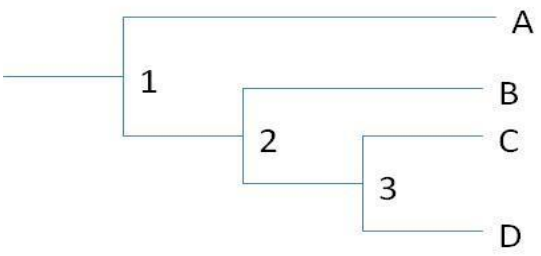


Figure 2: Phylogenetic tree example.

The phylogenetic tree or cladogram will consist of 'nodes' that indicate a common ancestor at the time of a divergence between groups of organisms. In Figure 2, node 1 represents ancestral divergence between species 'A' and the other three species, node 2 represents ancestral divergence between species 'B' and the two species 'C' and 'D', and node 3 represents common ancestry at the time of

divergence between species ‘C’ and ‘D’. According to the concept of phylogeny trees, species ‘A’ would have the fewest shared, derived traits with the other species and the sister taxons ‘C’ and ‘D’ would have the most shared traits. Sister taxons (or sister groups) are those which share the most traits within a phylogeny tree (also called a cladogram) relative to other groups or species within the tree. 'Relative' is the key word here since cladograms normally do not include all possible species in the evolution of lineages represented. So some sister taxons could be very close in evolutionary divergence and while others may actually be more distant than the tree may suggest. Traits that could be used to identify similarities include morphological, anatomical, physiological, and biochemical characteristics. The biochemical characteristics include the genome and proteome.

Read the papers from these links to identify the best way(s) to determine the phylogeny of selected members of the coronavirus family (*Coronaviridae*).

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7150129/

https://www.ebi.ac.uk/training/online/courses/introduction-to-phylogenetics/what-is-phylogenetics/

https://www.pnas.org/doi/10.1073/pnas.0501984102

1. What is the difference between phylogenetics and phylogenomics?
2. Do you think building a phylogenetic tree for members of the Coronoviridae using physical/morphological structures would be effective in producing a tree with a high level of confidence? Explain.

Interactive Activity: Tracking the origin of human SARS-CoV-2 using phylogenetic trees

Did the SARS-CoV-2 virus mutate in a novel way and jump from animals to humans (zoonotic)? Or did the virus mutate from established human viruses like SARS or MERS, both also being corona viruses, possibly still in a zoonotic fashion? Bats are a well-known zoonotic source for viral jumps to humans. They are not the only animal that may be involved in the transition. In fact, there may be intermediary animals that get a version of the virus from bats and then pass it

on to humans. Civet cats passed SARS CoV to humans and camels passed MERS CoV to humans.

Develop a hypothesis that may explain how humans acquired SARS CoV 2, then test the hypothesis using the materials and methods provided.

1. Open https://www.ncbi.nlm.nih.gov/, which is the NCBI site.
2. Look at the 'accession numbers' below (and what each represents):

NC 006577.2 (Human coronavirus HKU1)
NC 019843.3 (MERS CoV Human)
NC 004718.3 (SARS CoV Human)
NC 045512.2 (SARS CoV 2 Human)
MT121216.1 (Pangolin coronavirus)
ON026022.1 (SARS CoV 2 Omicron variant)
MN996532.1 (Bat RaTG13 coronavirus)

3. Search for the genome sequence of each of these on the NCBI website and copy to a new file:
    a. Keep the data base as "All Databases"
    b. Copy an accession number (but not what it represents) and paste it in the search box. Then click 'search.'
    c. The data base page will appear with a listing for a nucleotide sequence with the title for that accession. The title itself is a link to the nucleotide sequence. Click on that link to bring up the Genbank page. If there is an updated page link, click on that to get the latest Genbank page for that accession.
    d. Below the title is the term 'FASTA'. Click on that link to reveal the entire nucleotide sequence of the genome.
    e. Highlight the entire genome including the '> Genbank number and description' just above the nucleotides. Copy and paste into some type of text file.
    f. Repeat for all accessions. Save the file when finished.

g. Hint: the accession number is at the front of the sequence, You can put an easier identifier right after the ' > ' that will show up when you run the Clustal Omega program, Example: replace ' > NC 045512.2 etc. etc. ' with ' >SARSCoV2 (space) NC 045512.2 (space) (pertinent descriptors) '. By doing this,only 'SARSCoV2 ' will appear in the results for this accession. Note that ONLY what appears prior to the first space will be used as the identifier.

4. Now open https://www.ebi.ac.uk/Tools/msa/clustalo/, which is the Clustal Omega tool page.
5. Highlight your text file (you can use 'Select All' to do this), then copy the file.
6. Make sure you select 'DNA' as the set you are pasting, then paste the copied file in.
7. Make sure your output format is set to 'ClustalW with character counts.'
8. Click 'submit' and be patient; it is a large file and will take a bit of time to finish. As you are waiting, read further because you are not yet finished with this program.

When the Results finally appear, you should look at three of them in the following order: the Guide Tree, Phylogenetic Trees, and Results Summary/Percent Identity Matrix.

The Guide Tree provides an idea of a crude phylogeny of the SARS-CoV-2 virus. It provides some concept of distance in genomes for the different sources.

The Phylogenetic Tree uses the information gleaned from the Guide Tree and computes it in more powerful ways in order to get a tree that is usually the most parsimonious (i.e., Occam's Razor). Either tree will give you relationships, but the Phylogenetic Tree provides more confidence. Both should share some, but not all relationships. ClustalW uses the Neighbor-joining parameter in its algorithm to generate the tree. It assumes the tree is unrooted; having no common ancestor. In the broad sense our tree is rooted as these viruses are all betacoronaviruses. Moreover, the initially generated tree does not reflect published relationships. **Therefore, you must run a Simple Phylogeny.**

9. When the Job Results page appears for your initial submission, click on the 'Results Viewers' tab. Scroll to the bottom of the page and find 'Send to Simple Phylogeny' and click on it. The 'Simple Phylogeny' page appears.

a. Step1 of that page should show your original Clustal job number has already been entered.
b. In Step 2, the two options you need to change are:
    i. 'Clustering Method' - change it from 'Neighbor-joining' to 'UPGMA'
    ii. Change 'P.I.M.' to 'on'. (P.I.M.=percent identity matrix)
c. Click on 'submit'. The phylogeny cladogram and the PIM should arrive soon.

The Percent Identity Matrix determines exactly how similar the different genomes are to each other.

If you transpose the accessions numbers in the matrix horizontally as well as vertically you have what is sometimes called a 'look-up table' or a 'triangular matrix'. The vertical and horizontal intersection of the same accession number will yield a value of 100%. The vertical and horizontal intersection of two different accessions will yield a percentage of how identical they are: see https://home.cc.umanitoba.ca/~frist/PLNT7690/lec04/lec04.1.html

Assessment Activity:

Answer the following questions:

1. Did the results of the cladogram and the PIM you generated support or refute your hypothesis regarding the possible origin of SARS CoV 2? Explain.
    a. To support your answer, include a screenshot of your phylogram as a Figure and your PIM results as a Table. Be sure each has a title. Refer to these in answering the question.
2. In order to have clarification of the origins of SARS CoV 2 what type of data would need to be collected? The following article link may help. https://www.sciencedirect.com/science/article/pii/S0092867421009910
3. Genetic variability in sexually reproducing organisms occurs in a number of ways. Random fertilization within the population; during production of gametes (sperm and egg), independent assortment of the sets of chromosomes previously inherited is a big contributor. Switching sections between chromosome pairs of the sets during meiosis is known as genetic recombination and also contributes to variation. Mutations along the

nucleotide sequence of the genome are the original source of variation and occur in all organisms. How do you think coronavirus particles (the viruses) obtain variation? Support your answer.

a. Try answering using your own background knowledge, then check your ideas with findings from the following linked article: https://journals.asm.org/doi/10.1128/JVI.01031-17#F1

References:

Holmes, Kathryn V. “CORONAVIRUSES (CORONAVIRIDAE).” Encyclopedia of Virology (1999): 291–298. doi:10.1006/rwvi.1999.0055

Phylogenetics: An Introduction (Online Tutorial). EMBL-EBI Training, 2022. 10.6019/TOL.phyl.2015.00001.1

Rajagopalan, Maanasa. "Knowing Our Rival–Coronaviridae: The Virus Family". Fighting the COVID-19 Pandemic, edited by Manal Baddour, IntechOpen, 2021. 10.5772/intechopen.98806.

Pharmacology, Part 1 – Review of Pertinent Topics – High School, Informal Learners, and Non-Science Major College Freshman

Learning Outcomes, Part 1:

- Describe the members and properties of the members of the coronavirus family
- Describe the key steps in the SARS-CoV-2 lifecycle
- Identify the phases of a clinical trial
- Understand the importance of a clinical trial for development of Pharmacologic interventions for COVID -19

Background Lesson and Reading, Part 1:

The SARS-CoV-2 virus is a member of the coronavirus family. These viruses cause mild to moderate upper respiratory tract illnesses. There have been several members of the coronavirus family that have emerged from animal reservoirs over the last 20 years that have caused serious, widespread illness and death. These include SARS-CoV which was identified in 2002 and MERS-CoV which was identified in September 2012. Most recently has been the emergence of SARS-CoV-2 in December of 2019 in Wuhan, China. The SARS-CoV-2 virus is an RNA virus that attached to and enters the cells of the upper respiratory tract utilizing the ACE 2 protein receptor. Once inside the cell, the virus utilizes the components of the host cell to make more virus particles.

Interactive Activity, Part 1:

After watching the following video, please answer the questions about Coronavirus and SAR-CoV-2.

Video: Lifecycle of the Coronavirus

1. SARS-CoV-2 is a member of the coronavirus family. What is a coronavirus?
2. What are some other members of the coronavirus family?

3. What are the key steps in the SARS-CoV-2 lifecycle?

Assessment Activity, Part 1:

Assessment type 1:

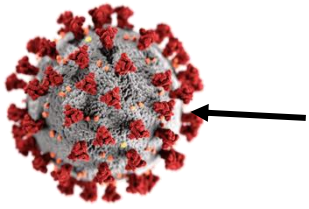
https://quizizz.com/admin/presentation/61cf0e0994c352001db0da61

Assessment type 2:

1. SARS-CoV-2 attaches to this surface protein on a respiratory cell.
    a. *ACE-2 protein*
    b. Insulin protein
    c. Spike protein
    d. Angiotensin I

2. Which of the following are examples of a coronavirus?
    a. *MERS*
    b. *SARS-CoV-2*
    c. Rubella
    d. Influenza

3. What are the purpose of the spikes found on the surface of the coronavirus?



    a. *Attach to the host cell*
    b. Encase the RNA of the virus
    c. Replicate the virus
    d. Allow the virus to leave the cell
4. Draw and label the main steps of the SARS-CoV-2 lifecycle.

Pharmacology, Part 2 - Clinical Trials and Drug Repurposing – High School, Informal Learners, and Non-Science Major College Freshman

Learning Outcomes, Part 2:

- Describe the phases of a clinical trial
- Discuss the importance of a clinical trial

Background Lesson and Reading, Part 2:

Clinical studies are done for a variety of reasons. It could be to investigate a disease, test a treatment or learn how a certain behavior affects people's health. There are two types of clinical studies that are routinely conducted, observational studies and clinical trials. In an observational study, researchers are not conducting experiments or testing a treatment. In this type of study researchers are trying to understand a situation and develop a hypothesis that can be tested in a clinical study. While an observational study cannot prove that one thing causes another, it can find associations between things. Some of the examples of observational studies are cohort studies, ecological studies or case studies. A clinical trial allows researchers to test new methods of preventing, detecting or treating a disease or to improve quality of life for patients that may have a chronic condition. Clinical trials are conducting in four phases each of which has a specific goal.

Interactive Activity, Part 2:

After reviewing the following website, please answer the questions regarding clinical studies:
Web site 1: https://clinicaltrials.gov/ct2/about-studies/learn

Web site 2:https://www.fda.gov/patients/drug-development-process/step-3-clinical-research

1. What is a clinical trial?
2. Clinical trials for drug development are described by phases. What are these phases?
3. What is the importance of clinical trials?

Assessment Activity, Part 2:

1. True or False. An observational study is conducted to test a treatment for a disease.
2. Which of the following would be conisistent with a phase 3 of a clinical trial.
    a. Last phase of trial that typically enrolls several thousand volunteers that have suffer from the disease in question.
    b. Initially begins with a small number of healthy volunteers and lasts a few months
    c. Lasts 1 to 4 years with 300-3000 participants that have the disease in question
    d. None of the above
3. Explain why clinical trials are important.

Pharmacology, Part 3: Pharmacology of COVID-19 – High School, Informal Learners, and Non-Science Major College Freshman

Learning Outcomes, Part 3:

- Discuss the repurposing of specific drugs for use with COVID patients
- Discuss the different methods for production of a SARS-CoV-2 vaccine
- Identify the therapeutic class of a COVID treatment
- Identify if a drug was recommended for use with COVID patients
- Explain the difference in FDA approval of a treatment and Emergency Use Authorization of a treatment

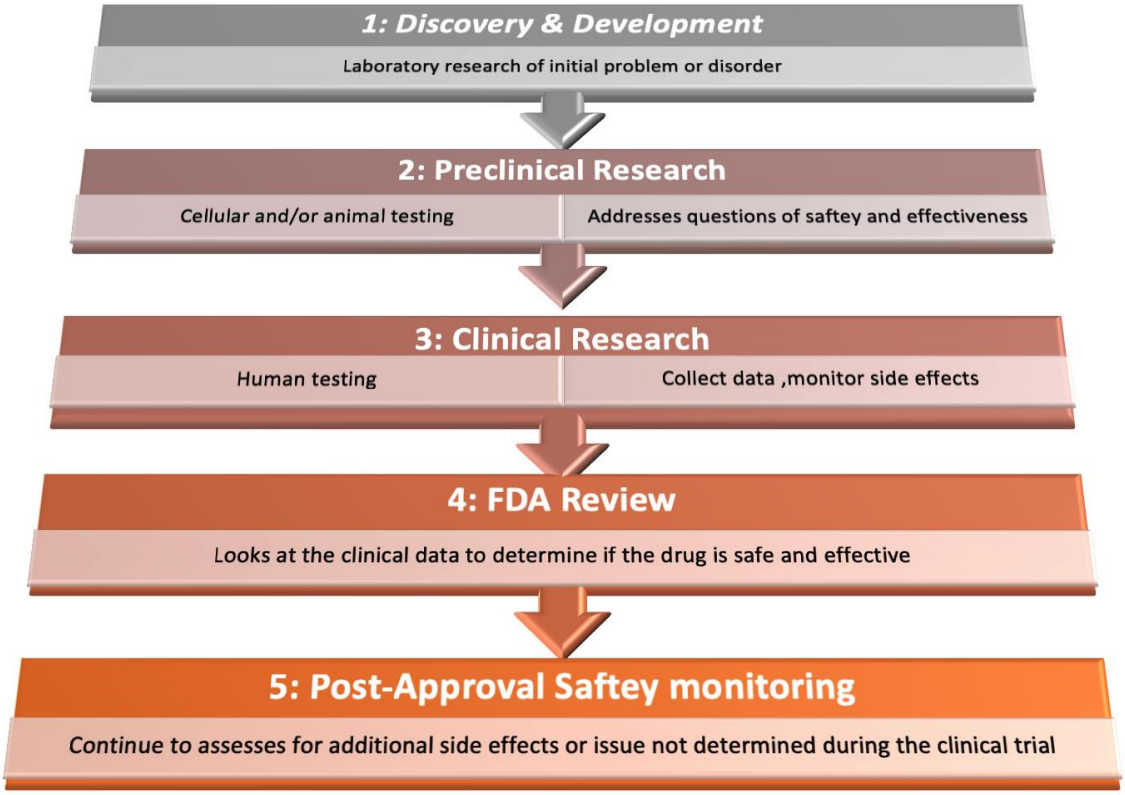
Background Lesson and Reading, Part 3:

Since the initial outbreak of SARS-CoV-2 in December of 2019, there has been a great deal of research to understand the virus lifecycle, potential treatment approaches and pharmacological interventions. There have been a several vaccine strategies that have produced safe and effective vaccines and a variety of drugs have received Emergency use authorization (EUA) for treatment of patients with COVID-19. An emergency use authorization (EUA) allows the FDA to facilitate the use of medical countermeasure during a public health crisis such as the SARS-CoV-2 pandemic.

SARS-CoV-2 was a novel virus with little known about its mechanism of action and no approved treatments for the associated condition, COVID-19. The FDA created a special emergency program, CTAP (Coronovirus Treatment Acceleration Program), that used every available method to identify and initiate new treatments to patient as quickly as possible. As a part of this program, data is collected to determine the efficacy of a specific treatment and any potentially harmful side effects. Some of the drugs that have been investigated are antivirals, immunonodulators and neutralizing antibody therapies. Some of these drugs have been identified as appropriate treatments for COVID-19 and some have been shown to not be a viable treatment method.

There were no therapeutic interventions for the disease, COVID-19, that resulted from infection from the SARS-CoV-2 virus. Normally, new drug therapies go through a series of five steps in the drug development process.

Figure 1: Drug development process



There were no drugs already developed for COVID-19, so the practice of repurposing drugs was used to quickly determine effective drug treatments that were originally utilized for other conditions. Many of the drugs currently used for the treatment of COVID-19 were identified using this method. The drugs that were identified as possible treatments for COVID-19 went through the drug development process for use with another condition. Additional data on drug efficacy with COVID-19 patients was collected during use of the treatment to determine if the treatment was appropriate, safe and effective for use with these patients.

Interactive Activity, Part 3:

Utilizing the following websites for information, please complete the following questions:

Ted Talk – Drug Repurposing

The Race for Corovavirus Vaccines: A Graphical Guide

NIH – COVID-19 Therapies

Understanding Regulatory Terminology

1. What is Drug Repurposing and why was this an advantage with COVID-19 treatments
2. What different methods of vaccine development were utilized to make a vaccine for SARS-CoV-2? Which methods were most effective and why?
3. Please classify the following drug therapies for COVID-19 according to the following categories: Anti-virals or immune modulators
    a. Remdesivir
    b. Dexamethasone
    c. Hydroxychloroquine
    d. Lopinavir
    e. Ivermectin
4. Were the Drug Therapies from question 3 approved for use for COVID-19. If not, could you explain why.
5. Explain the following terms:
    a. FDA approved
    b. Emergency Use Authorization (EUA)
    c. Investigational Treatments
    d. Expanded Access

Assessment Activity, Part 3:

Complete the following chart:

Drug Therapy	Drug Mechanism	FDA Approval for Use
Remdesivir		
Ivermectin		
Hydroxychloroquine		
Dexamethasone		

Pharmacology, Part 1: Review of Pertinent Topics: Pharmacology of COVID-19 – Non-Science Major and Lower-Level College Students

Learning Outcomes, Part 1:

- Compare the members and properties of the members of the coronavirus family
- Summarize the steps in the SARS-CoV-2 lifecycle
- Discuss the method of entry of the SARS-CoV-2 virus into the cell and the importance of the ACE-2 protein in this process
- Summarize the effect of mutations of the spike protein on SARS-CoV-2 host cell interactions
- Describe the phases of a clinical trial
- Recognize the importance of a clinical trial in the development of Pharmaceutical interventions for COVID-19

Background Lesson and Reading, Part 1:

The SARS-CoV-2 virus is a member of the coronavirus family. These viruses have been idetified to cause mild to moderate upper respiratory tract illnesses. There have been several members of the coronavirus family that have emerged from animal reservoirs over the last 20 years that have caused serious, widespread illness and death. These include SARS-CoV which was identified in 2002 and MERS-CoV which was identified in September 2012. Most recently has been the emergence of SARS-CoV-2 in December of 2019 in Wuhan, China.

The SARS-CoV-2 virus is an RNA virus that attached to and enters the cells of the upper respiratory tract utilizing the ACE 2 proteins. Once inside the cell, the virus utilizes the components of the host cell to make more virus particles. The spike protein is a key viral structure in the attachement and entry of the virus into the host cell. Additionally, vaccines like the Moderna or Pfizer RNA vaccine utilize the spike protein to produce antibodies and development immunity to the virus. The spike protein and mutation of this viral structure have been an area of concern in the design and implementation of the COVID vaccines.

Interactive Activity, Part 1:

After watching the following videos, please answer the questions about Coronavirus Structure and Lifecycle.

Video 1: Lifecycle of the Coronavirus

Video 2: Key Stages of SARS-CoV-2 Lifecycle

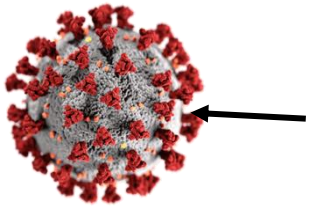
1. Discuss the classification of SARS-CoV-2 and the other members of coronavirus family
2. Discuss the key steps of the SARS-CoV-2 lifecycle.
3. What is the importance of ACE-2 protein?
4. How does mutation of the spike protein of SARS-CoV-2 impact interactions with the host cells?
5. What method of entry into the cell is used by the coronavirus?

Assessment Activity, Part 1:

1. SARS-CoV-2 attaches to this surface protein on a respiratory cell.
   a. *ACE-2 protein*
   b. Insulin protein
   c. Spike protein
   d. Angiotensin I

2. Which of the following are examples of a coronavirus?
   a. *MERS*
   b. *SARS-CoV-2*
   c. Rubella
   d. Influenza

3. What is the purpose of the spikes found on the surface of the coronavirus?



   a. *Attach to the host cell*
   b. Encase the RNA of the virus
   c. Replicate the virus
   d. Allow the virus to leave the cell
4. Draw and label the main steps of the SARS-CoV-2 lifecycle.
5. Mutations in the spike protein can do all of the following except:
   a. Enhance the binding between the spike protein and the ACE-2 protein
   b. Alter binding of antibodies to the spike protein
   c. *Increase viability of virus outside the host*
   d. Alter efficacy of vaccine response
6. Explain the phases of a clinical trial.

Pharmacology, Part 2 - Clinical Trials and Drug Repurposing: Pharmacology of COVID-19 – Non-Science Major and Lower-Level College Students

Learning Outcomes, Part 2:

- Describe the phases of a clinical trial
- Compare a clinical study and observational study
- Discuss the importance of an IRB in a clinical study
- Describe the importance and process of drug repurposing

Background Lesson and Reading, Part 2:

Clinical studies are done for a variety of reasons. It could be to investigate a disease, test a treatment or learn how a certain behavior affects people's health. There are two types of clinical studies that are routinely conducted, observational studies and clinical trials. In an observational study, researchers are not conducting experiments or testing a treatment. In this type of study researchers are trying to understand a situation and develop a hypothesis that can be tested in a clinical study. While an observational study cannot prove that one thing causes another, it can find associations between things. Some of the examples of observational studies are cohort studies, ecological studies or case studies. A clinical trial allows researchers to test new methods of preventing, detecting or treating a disease or to improve quality of life for patients that may have a chronic condition. Clinical trials are conducting in four phases each of which has a specific goal. Because clinical trials are done with human subjects, appropriate steps must be taken to protein the rights and welfare of those participants. An Institutional Review Board (IRB) is a requirement under FDA regulations and requires the assembly of a group that is tasked with reviewing research proposals and monitoring any research involving human subjects. An IRB has the ability to approve, deny or require modifications to a research proposal submitted by the clinicians. Additionally, the IRB continues to monitor the research during the clinical trial to ensure the rights and welfare of the human subjects are protected.

Drug repurposing is a method of exploring the therapeutic benefits of existing drugs in clinical trials. This method streamlines the process of assessing the benefits of a specific drug for another condition and avoids some of the constraints of resources, time and financial considerations. Drug repurposing was a strategy used with treatment of COVID-19 to develop safe, effective therapeutic interventions that were already available.

Interactive Activity, Part 2:

After reviewing the following resources, please answer the questions regarding clinical studies:
Web site 1: https://clinicaltrials.gov/ct2/about-studies/learn
Web site 2: https://www.fda.gov/patients/drug-development-process/step-3-clinical-research
Web Site 3: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/institutional-review-boards-frequently-asked-questions

YouTube video: https://www.youtube.com/watch?v=5olSLe4faiw

1. What is a clinical trial?
2. What is the difference between a clinical trial and an observational study?
3. Clinical trials for drug development are described by phases. What are these phases?
4. What is an IRB and what is its importance in the process of clinical trials?
5. What is drug repurposing?

Assessment Activity, Part 2:

1. True or False. An observational study is conducted to test a treatment for a disease.
2. Which of the following would be conisistent with a phase 3 of a clinical trial.
    a. Last phase of trial that typically enrolls several thousand volunteers that have suffer from the disease in question.

b. Initially begins with a small number of healthy volunteers and lasts a few months
c. Lasts 1 to 4 years with 300-3000 participants that have the disease in question
d. None of the above

3. Explain why clinical trials are important.

4. Which of the following is NOT true of an Instituitional Review Board (IRB)
    a. Provides a peer review of the clinical trial data
    b. Required under FDA regulations
    c. Ensures the rights and welfare of the human subjects in a clinical trial
    d. Can require modifications to a submitted research proposal

Discuss the advantages and disadvantages of drug repurposing for COVID-19.

Pharmacology, Part 3: Pharmacology of COVID-19 – Non-Science Major and Lower-Level College Students

Learning Outcomes, Part 3:

- Discuss the repurposing of specific drugs for use with COVID patients
- Identify the therapeutic class of a COVID treatment
- Identify if a drug was recommended for use with COVID patients and what clinical data was used to make that decision
- Identify if a drug was not recommended for use with COVID patients and what clinical data was used to make that decision
- Identify the original use of potential treatments for use with COVID patients
- Discuss the different methods for production of a SARS-CoV-2 vaccine
- Compare and contrast FDA approval of a treatment and Emergency Use Authorization

Background Lesson and Reading, Part 3:

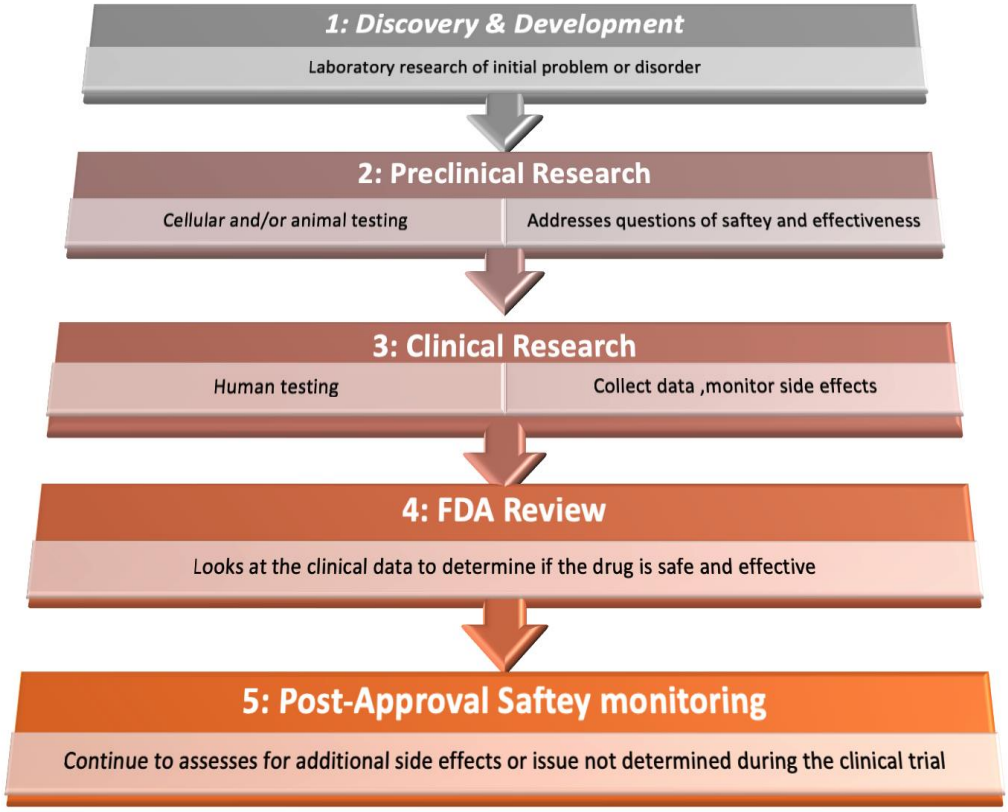
Since the initial outbreak of SARS-CoV-2 in December of 2019, there has been a great deal of research to understand the virus lifecycle, potential treatment approaches and pharmacological interventions. There have been a several vaccine strategies that have produced safe and effective vaccines and a variety of drugs have received Emergency use authorization (EUA) for treatment of patients with COVID-19. An emergency use authorization (EUA) allows the FDA to facilitate the use of medical countermeasure during a public health crisis such as the SARS-CoV-2 pandemic.

SARS-CoV-2 was a novel virus with little known about its mechanism of action and no approved treatments for the associated condition, COVID-19. The FDA created a special emergency program, CTAP (Coronovirus Treatment Acceleration Program), that used every available method to identify and initiate new treatments to patient as quickly as possible. As a part of this program, data is collected to determine the efficacy of a specific treatment and any potentially harmful side effects. Some of the drugs that have been investigated are antivirals, immunonodulators and neutralizing antibody therapies. Some of these drugs

have been identified as appropriate treatments for COVID-19 and some have been shown to not be a viable treatment method.

There were no therapeutic interventions for the disease, COVID-19, that resulted from infection from the SARS-CoV-2 virus. Normally, new drug therapies go through a series of five steps in the drug development process.

Figure 1: Drug development process



There were no drugs already developed for COVID-19, so the practice of repurposing drugs was used to quickly determine effective drug treatments that were originally utilized for other conditions. Many of the drugs currently used for the treatment of COVID-19 were identified using this method. The drugs that were identified as possible treatments for COVID-19 went through the drug development process for use with another condition. Additional data on drug efficacy with COVID-19 patients was collected during use of the treatment to

determine if the treatment was appropriate, safe and effective for use with these patients.

Interactive Activity, Part 3:

Utilizing the following websites for information, please complete the following questions:

The Race for Corovavirus Vaccines: A Graphical Guide

NIH – COVID-19 Therapies

Understanding Regulatory Terminology

COVID-19: Characteristics and Therapeutics

1. What different methods of vaccine development were utilized for SARS-CoV-2? Which methods were most effective and why?
2. Please fill out the table for the following treatments for COVID-19. Indicate the original use of the treatment, the mechanism of action, if the therapy was approved by the FDA and any interaction or precautions.

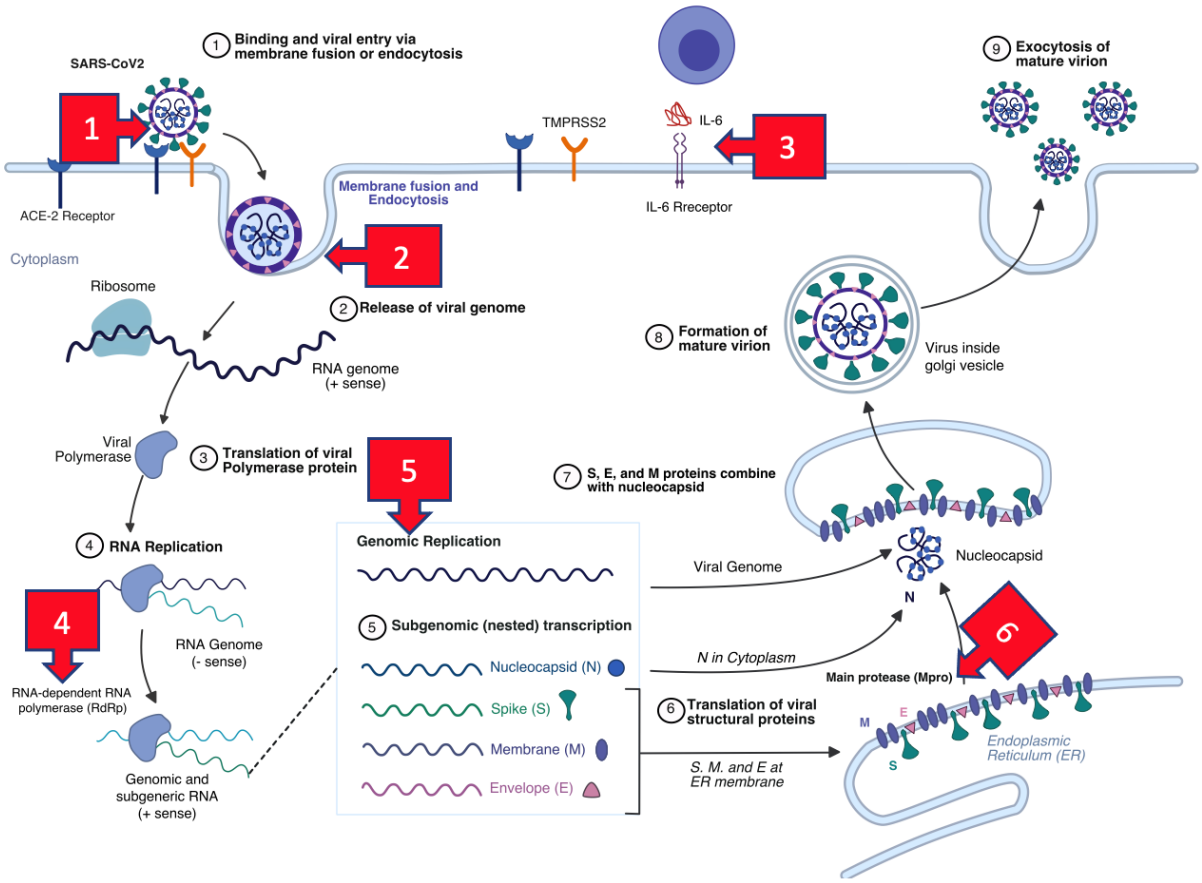
Drug Therapy	Original use	Mechanism of action	FDA approval status	Interactions/ Precautions
Remdesivir				
Favipiravir				
Tocilizumab				
Dexamethasone				
Chloroquine/ Hydroxychloroquine				
Lopinavir				
Ivermectin				
Molnupiravir				
Bamlanivimab/ Etesevimab				

3. Compare and contrast FDA approval of a therapy and Emergency Use Authorization (EUA).

Assessment Activity, Part 3:

Using the graphic below, identify which part of the SARS-CoV-2 lifecycle the following therapies target:

- Remdesivir
- Tocilizumab
- Hydroxychloroquine
- Lopinavir
- Bamlanivimab
- Molnupiravir



Pharmacology, Part 1: Pharmacology of COVID-19, Review of Pertinent Topics – Science Major and Upper-Level College Students

Learning Outcomes, Part 1:

- Discuss the properties and classification of the coronavirus family
- Outline the steps of the SARS-CoV-2 lifecycle and highlight the importance of the ACE-2 protein
- Explain the effect of mutations to the spike protein and how it impacts viral entry.
- Analyze the effect of SARS-CoV-2 to the RAAS system and identify the effect of ACE inhibitors to the risk of COVID-19 infection.
- Apply the variables of viral load and immune system integrity on disease progression.
- Evaluate the connection between Angiotensin II/Angiotensin and patient susceptibility to COVID infection
- Outline the phases and timeframe for drug development
- Identify the process to fast track for clinical trials
- Examine the advantages of drug repurposing for COVID -19

Background Lesson and Reading, Part 1:

The SARS-CoV-2 virus is a member of the coronavirus family. These viruses have been identified to cause mild to moderate upper respiratory tract illnesses. There have been several members of the coronavirus family that have emerged from animal reservoirs over the last 20 years that have caused serious, widespread illness and death. These include SARS-CoV which was identified in 2002 and MERS-CoV which was identified in September 2012. Most recently has been the emergence of SARS-CoV-2 in December of 2019 in Wuhan, China.

The SARS-CoV-2 virus is an RNA virus that attached to and enters the cells of the upper respiratory tract utilizing the ACE 2 proteins. Once inside the cell, the virus utilizes the components of the host cell to make more virus particles. The spike protein is a key viral structure in the attachement and entry of the virus into the host cell. Additionally, vaccines like the Moderna or Pfizer RNA vaccine utilize the

spike protein to produce antibodies and development immunity to the virus. The spike protein and mutation of this viral structure have been an area of concern in the design and implementation of the COVID vaccines.

The renin-angiotensin system is an important regulatory system in the body. The SARS-CoV-2 virus utilizes the ACE-2 receptor protein to enter the respiratory cells. The ACE-2 protein is an important regulator as a counter balance to the Renin-Angiotensin system. Some research has focused on patients taking ACE inhibitors or Angiotensin receptor blockers and the potential that these drugs could increase risk of viral infection by upregulation of the ACE-2 proteins. The other considerations that are important in assessing risk of infection by the virus are initial viral load, integrity of the immune system as well as the number and configuration of the ACE-2 proteins.

After watching the following videos, please answer the questions about Coronavirus Structure and Lifecycle.

Video 1: Lifecycle of the Coronavirus

Video 2: Key Stages of SARS-CoV-2 Lifecycle

1. Discuss the classification of SARS-CoV-2 and the other members of coronavirus family
2. Discuss the key steps of the SARS-CoV-2 lifecycle.
3. What is the importance of ACE-2 protein?
4. How does mutation of the spike protein of SARS-CoV-2 impact interactions with the host cells?
5. What method of entry into the cell is used by the coronavirus?

Interactive Activity, Part 1:

After watching the following video, please answer the questions about the RAAS pathway and Coronavirus.

Video: COVID and the ACE-2 Surface Protein

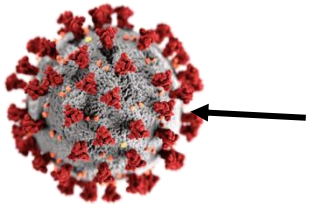
1. Describe the components of the Renin-Angiotensin-Aldosterone System (RAAS).
2. What is the role of the ACE-2 protein in RAAS?
3. Compare the effect of Angiotensin II and Angiotensin (1-7)
4. Explain the function of ACE inhibitors and Angiotensin Receptor Blockers in patients with high blood pressure. Do the use of these drugs increase risk of COVID-19 infection?
5. Describe how the variables of initial viral load, immune system integrity and number/configuration of ACE-2 proteins can potential affect COVID-19 disease progression.
6. Discuss the potential connection between Angiotensin II/Angiotensin (1-7), the baseline health of a patient and their susceptibility to COVID infection.

Assessment Activity, Part 1:

1. SARS-CoV-2 attaches to this surface protein on a respiratory cell.
    a. *ACE-2 protein*
    b. Insulin protein
    c. Spike protein
    d. Angiotensin I

2. Which of the following are examples of a coronavirus?
    a. *MERS*
    b. *SARS-CoV-2*
    c. Rubella
    d. Influenza

3. What are the purpose of the spikes found on the surface of the coronavirus?



    a. *Attach to the host cell*
    b. Encase the RNA of the virus

c. Replicate the virus
d. Allow the virus to leave the cell

4. Draw and label the main steps of the SARS-CoV-2 lifecycle.

5. Mutations in the spike protein can do all of the following except:
    a. Enhance the binding between the spike protein and the ACE-2 protein
    b. Alter binding of antibodies to the spike protein
    c. Increase viability of virus outside the host
    d. Alter efficacy of vaccine response

6. Explain the phases of a clinical trial.

7. You are assessing two patients, one with mild COVID, the other with severe COVID. Describe how the variables of viral load, baseline health of the patient and use of drugs such as ACE inhibitors could affect the presentation and level of severity of COVID.

Pharmacology, Part 2 - Clinical Trials and Drug Repurposing: Pharmacology of COVID-19 – Science Major and Upper-Level College Students

Learning Outcomes, Part 2:

- Describe the phases of a clinical trial
- Discuss the importance of an IRB in a clinical study
- Discuss the importance and process of fast track in drug development
- Compare traditional vaccine development with the COVID vaccine development
- Describe the importance and process of drug repurposing

Background Lesson and Reading, Part 2:

Clinical studies are done for a variety of reasons. It could be to investigate a disease, test a treatment or learn how a certain behavior affects people's health. There are two types of clinical studies that are routinely conducted, observational studies and clinical trials. In an observational study, researchers are not conducting experiments or testing a treatment. In this type of study researchers are trying to understand a situation and develop a hypothesis that can be tested in a clinical study. While an observational study cannot prove that one thing causes another, it can find associations between things. Some of the examples of observational studies are cohort studies, ecological studies or case studies. A clinical trial allows researchers to test a new method of preventing, detecting or treating a disease or to improve quality of life for patients that may have a chronic condition. Clinical trials are conducting in four phases each of which has a specific goal. Because clinical trials are done with human subjects, appropriate steps must be taken to protein the rights and welfare of those participants. An Institutional Review Board (IRB) is a requirement under FDA regulations and requires the assembly of a group that is tasked with reviewing research proposals and monitoring any research involving human subjects. An IRB has the ability to approve, deny or require modifications to a research proposal submitted by the

clinicians. Additionally, the IRB continues to monitor the research during the clinical trial to ensure the rights and welfare of the human subjects are protected.

Drug repurposing is a method of exploring the therapeutic benefits of existing drugs in clinical trials. This method streamlines the process of assessing the benefits of a specific drug for another condition and avoids some of the constraints of resources, time and financial considerations. Drug repurposing was a strategy used with treatment of COVID-19 to develop safe, effective therapeutic interventions that were already available.

Interactive Activity, Part 2:

Utilizing the following resources, please answer the questions regarding clinical studies and drug repurposing:

Clinical Trials
Drug Development Process
Fast Track Drug Approval
Developing COVID-19 Vaccines at Pandemic Speed
TED Talk – Drug Repurposing
Challenges for Drug Repurposing in the COVID-19 Pandemic Era

1. Clinical trials for drug development are described by phases. What are these phases? What is the typical timeframe for drug development?
2. What is an IRB and what is its importance in the process of clinical trials?
3. What is the process of fast track and why is it used?
4. Compare the traditional vaccine development with the COVID vaccine development.
5. What is drug repurposing? What are the advantages and challenges of drug repurposing with COVID? What are some of the drugs that have been identified for repurposing for COVID?

Assessment Activity, Part 2:

1. True or False. An observational study is conducted to test a treatment for a disease.
2. Which of the following would be conisistent with a phase 3 of a clinical trial.

a. Last phase of trial that typically enrolls several thousand volunteers that have suffer from the disease in question.
b. Initially begins with a small number of healthy volunteers and lasts a few months
c. Lasts 1 to 4 years with 300-3000 participants that have the disease in question
d. None of the above

3. Explain why clinical trials are important.

4. Which of the following is NOT true of an Instituitional Review Board (IRB)
    a. Provides a peer review of the clinical trial data
    b. Required under FDA regulations
    c. Ensures the rights and welfare of the human subjects in a clinical trial
    d. Can require modifications to a submitted research proposal

5. Discuss the advantages and disadvantages of drug repurposing for COVID-19.

5. Explain what conditions must be met for a drug to receive fast track designation.

Pharmacology, Part 3: Pharmacology of COVID-19 – Science Major and Upper-Level College Students

Learning Outcomes, Part 3:

- Determine the mechanism of action of a drug/treatment that may have been considered for use with COVID patients
- Assess the clinical information that determined if a treatment was recommended for use with COVID patients.
- Examine the original use of potential treatments for COVID patients and why it was repurposed for SARS-CoV-2
- Evaluate the different methods for production of a Coronavirus vaccine
- Compare and contrast FDA approval of a treatment and Emergency Use Authorization
- Discuss the necessity and strategies for clear communication of scientific information

Background Lesson and Reading, Part 3:

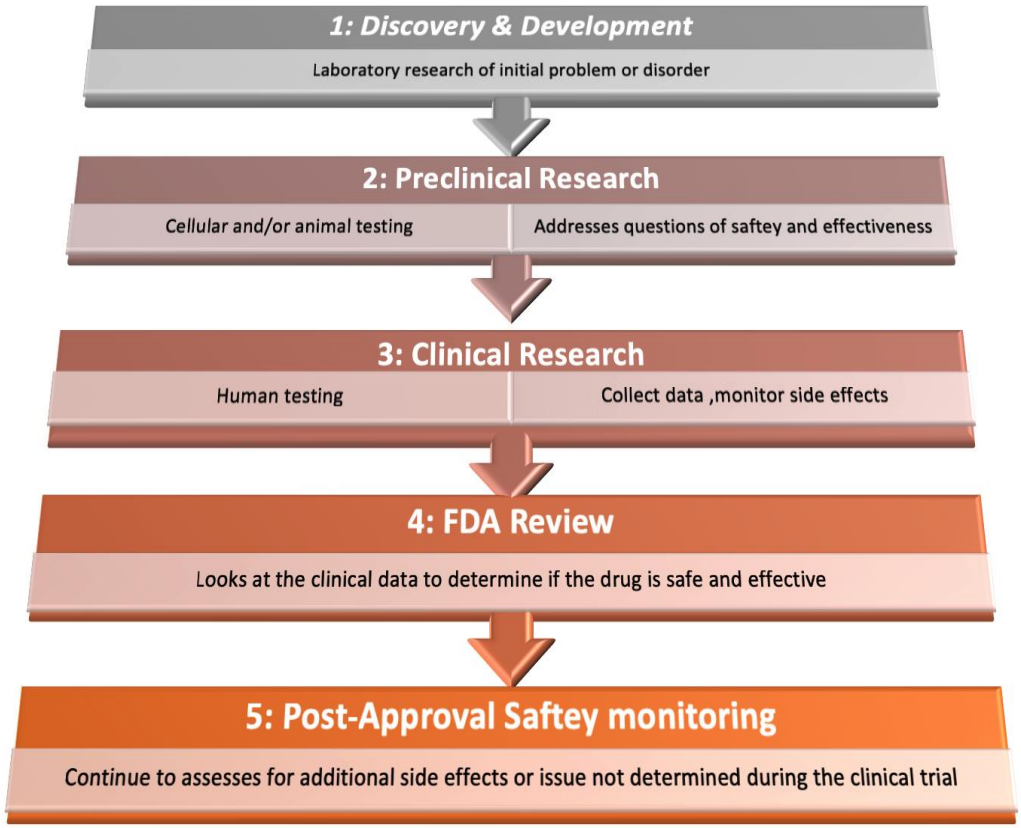
Since the initial outbreak of SARS-CoV-2 in December of 2019, there has been a great deal of research to understand the virus lifecycle, potential treatment approaches and pharmacological interventions. There have been a several vaccine strategies that have produced safe and effective vaccines and a variety of drugs have received Emergency use authorization (EUA) for treatment of patients with COVID-19. An emergency use authorization (EUA) allows the FDA to facilitate the use of medical countermeasure during a public health crisis such as the SARS-CoV-2 pandemic.

SARS-CoV-2 was a novel virus with little known about its mechanism of action and no approved treatments for the associated condition, COVID-19. The FDA created a special emergency program, CTAP (Coronovirus Treatment Acceleration Program), that used every available method to identify and initiate new treatments to patient as quickly as possible. As a part of this program, data is collected to determine the efficacy of a specific treatment and any potentially harmful side effects. Some of the drugs that have been investigated are antivirals,

immunonodulators and neutralizing antibody therapies. Some of these drugs have been identified as appropriate treatments for COVID-19 and some have been shown to not be a viable treatment method.

There were no therapeutic interventions for the disease, COVID-19, that resulted from infection from the SARS-CoV-2 virus. Normally, new drug therapies go through a series of five steps in the drug development process.

Figure 1: Drug development process



There were no drugs already developed for COVID-19, so the practice of repurposing drugs was used to quickly determine effective drug treatments that were originally utilized for other conditions. Many of the drugs currently used for the treatment of COVID-19 were identified using this method. The drugs that were identified as possible treatments for COVID-19 went through the drug development process for use with another condition. Additional data on drug efficacy with COVID-19 patients was collected during use of the treatment to

determine if the treatment was appropriate, safe and effective for use with these patients.

Interactive Activity, Part 3:

Utilizing the following websites for information, please complete the following questions:

The Race for Corovavirus Vaccines: A Graphical Guide

NIH – COVID-19 Therapies

Understanding Regulatory Terminology

COVID-19: Characteristics and Therapeutics

COVID-19 pandemic, infodemic and the role of eHealth literacy

1. What different methods of vaccine development were utilized for SARS-CoV-2? Which methods were most effective and why?
2. What implications could the development of an RNA vaccine have for other viruses such as HIV
3. Please fill out the table for the following treatments for COVID-19. Indicate the original use of the treatment, the mechanism of action, if the therapy was approved by the FDA and any interaction or precautions.

Drug Therapy	Original use	Mechanism of action	FDA approval status	Interactions/ Precautions
Remdesivir				
Favipiravir				
Tocilizumab				
Dexamethasone				
Chloroquine/ Hydroxychloroquine				
Lopinavir				

Ivermectin				
Molnupiravir				
Bamlanivimab/ Etesevimab				

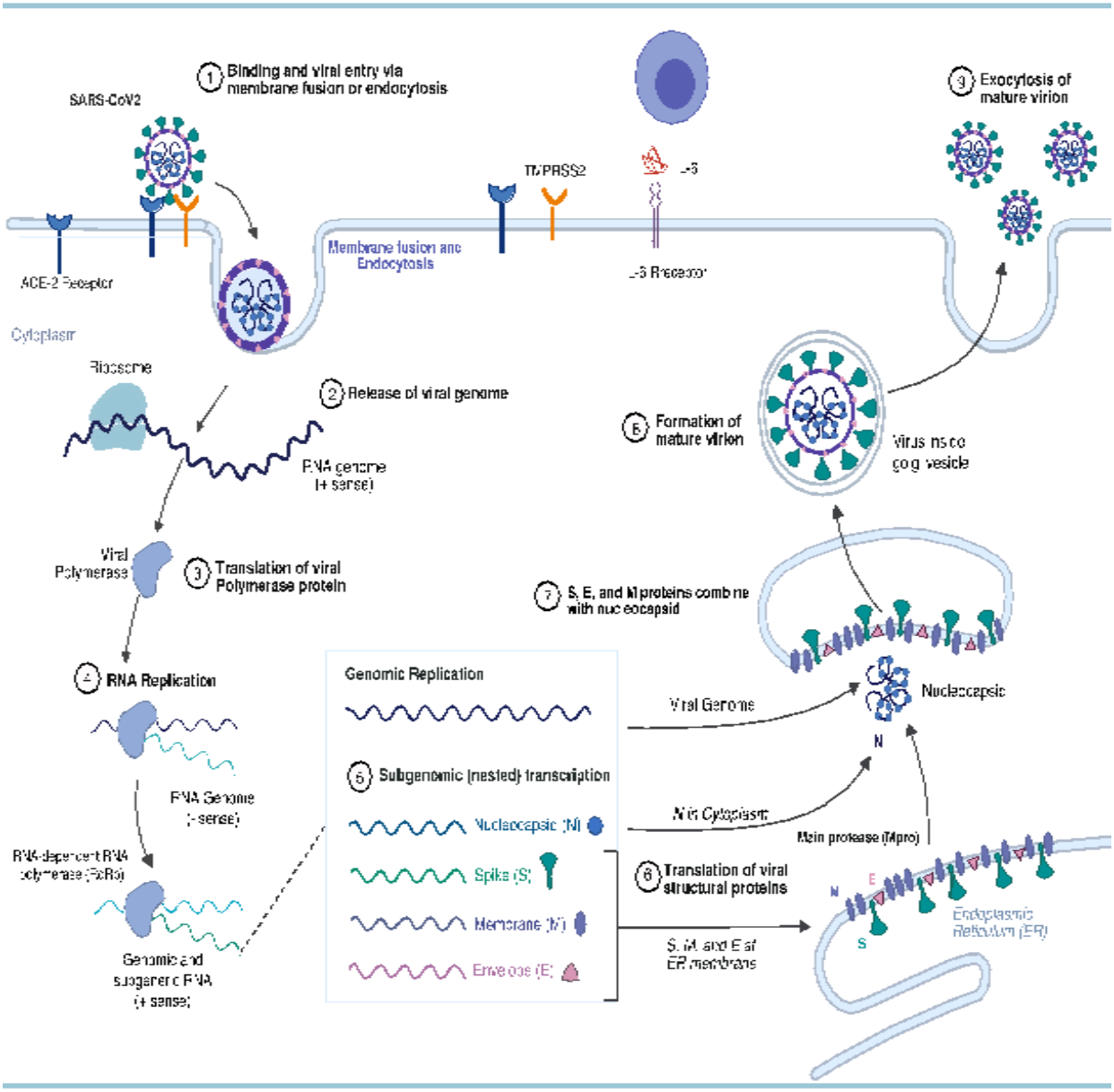
4. Compare and contrast FDA approval of a therapy and Emergency Use Authorization (EUA).

5. The Coronavirus pandemic has challenged us not only in the search for interventions but also with the flood of misinformation and misunderstanding of these treatments. Please choose two of the treatments in the above list that were not approved by the FDA and discuss the clinical data that lead to this decision. Also outline some of the common misconceptions that exist in the general public and how health professionals can help to combat this infodemic.

Assessment Activity, Part 3:

Using the graphic on the next page, identify what part of the SARS-CoV-2 lifecycle the following drugs therapies target:

Remdesivir, Tocilizumab, Hydroxychloroquine, Lopinavir, Bamlanivimab, Molnupiravir



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