COVID-19 qPCR Lab

Detecting SARS-CoV-2 Infection

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COVID-19 qPCR Lab
Detecting SARS-CoV-2 Infection
Instructor's and Student's Guide

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Student's Guide Contents

Background information

As a healthcare provider, your job is to diagnose your patients and provide the best treatment possible based on that diagnosis. However, it can be difficult to tell different illnesses apart because symptoms of many diseases are similar.

Today, you will test four travelers for COVID-19. As their healthcare provider, you'll use molecular techniques to determine whether your patients are infected with SARS-CoV-2 (pronounced "sars-co-vee-two"), the virus that causes the disease known as COVID-19.

SARS-CoV-2

Viruses are infectious agents with relatively simple structures: all viruses contain a small genome made of RNA or DNA surrounded by a coat made of protein and sometimes lipids (Figure 1). Because they must use a host's cellular machinery to reproduce, viruses are generally considered nonliving.

A virus must invade a living cell and use the cell's machinery to make copies of itself (Figure 2). Viruses recognize molecules on the outside of the host cell and then enter the cell. The SARS-CoV-2 virus is covered in specialized proteins called spike proteins that recognize and bind to specific receptors on human (and other mammalian) cells. This interaction between the virus and the host cell receptor triggers the entry of the virus into the cell.

Important acronyms

- **SARS-CoV-2:** severe acute respiratory syndrome coronavirus 2. A novel virus that emerged in human populations in 2019.
- **COVID-19:** coronavirus disease 2019, the disease resulting from infection with SARS-CoV-2 virus.

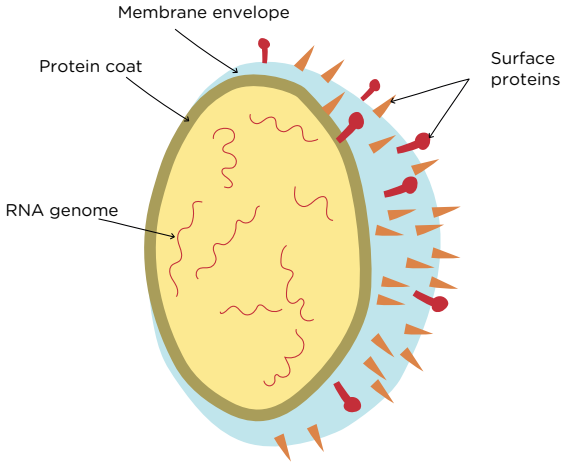


Figure 1. SARS-CoV-2 structure. The SARS-CoV-2 virus has an RNA genome packaged inside a protein coat and a membrane envelope. There are a handful of viral membrane surface proteins, including the spike protein. The spike protein binds with receptors on host cells to gain entry and begin the viral replication cycle.

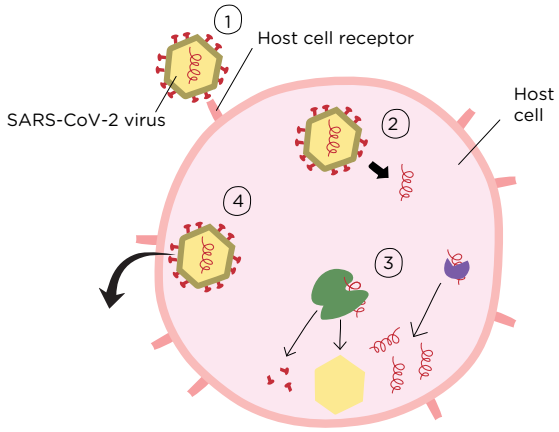


Figure 2. SARS-CoV-2 viral replication cycle. (1) The SARS-CoV-2 spike protein binds to host cell receptors and enters the cell. (2) The virus particle sheds its coat, releasing its RNA genome into the host cell. (3) The host cell copies the viral genome and expresses viral proteins, which come together to make new virus particles. (4) Newly assembled virus particles emerge from cell, ready to infect new host cells.

Once inside the cell, the viral genome is released from the protein coat, and the molecular machinery within the host cell manufactures viral proteins and replicates the viral genetic material. Newly made viral proteins and copies of the viral genome then come together to form new virus particles. These viruses emerge from the host cell, moving on to infect new cells—and new hosts if they're able to find their way out of the body, say through a cough or sneeze—and the cycle repeats itself.

While an infection can be disastrous for the host, the speed and efficiency of viral replication represent a success from the virus's point of view. Despite their small size, simple construction, and total dependence on living cells, viruses are remarkably efficient in achieving the primary functions of all biological entities: to persist and replicate.

Diagnosing SARS-CoV-2 infection

Different respiratory viruses that infect our airways often produce very similar symptoms, making it difficult to tell which virus underlies a person's particular illness. For example, a cold and a case of seasonal influenza may appear quite similar, both causing a cough, nasal congestion, and body aches. However, these diseases are caused by different viruses, and correctly diagnosing the infection can be important to treat the patient properly.

For COVID-19, quickly determining whether a person's symptoms are due to SARS-CoV-2 is essential for properly monitoring a person's disease and preventing further spread of the virus. While doctors use numerous lab tests to diagnose SARS-CoV-2 infection[1], we will focus on nucleic acid detection tests today.

Nucleic acid tests are used to diagnose many conditions, including both viral and bacterial infections. The key principle is that unique genetic sequences can be used to identify pathogens. For example, if genetic sequences that are unique to the SARS-CoV-2 virus are detected in a patient's sample, their doctor can diagnose them as being infected with that specific virus.

[1]For information comparing different COVID tests, refer to the *Additional background*, page 62.

A SARS-CoV-2 nucleic acid detection test typically involves the following three steps (Figure 3):

Step 1 - Collect patient sample:
For a respiratory virus like SARS-CoV-2, the nasal passage is typically swabbed to collect viral particles.

Step 2 - Extract genetic material:
The cells and virus particles are ruptured to release their DNA and RNA. Because SARS-CoV-2 is an RNA virus, scientists specifically isolate RNA from the sample. This will include any RNA present on the swab, and will likely include RNA from the patient and RNA from any bacteria or viruses present in the nasal passage. The detection of RNA viruses like SARS-CoV-2 requires an intermediate step where RNA is converted into DNA in a process called *reverse transcription*. Once the RNA has been converted into DNA, scientists attempt to copy one or more gene segments specific to the SARS-CoV-2 virus.

Step 3 - Amplify and analyze viral sequence:
Scientists commonly use a technique called polymerase chain reaction (PCR) to copy SARS-CoV-2 genetic sequences. If the PCR copies the gene, it means that the SARS-CoV-2 virus was present in the sample. If the PCR does not generate copies of the gene, it means that the SARS-CoV-2 virus was not present.

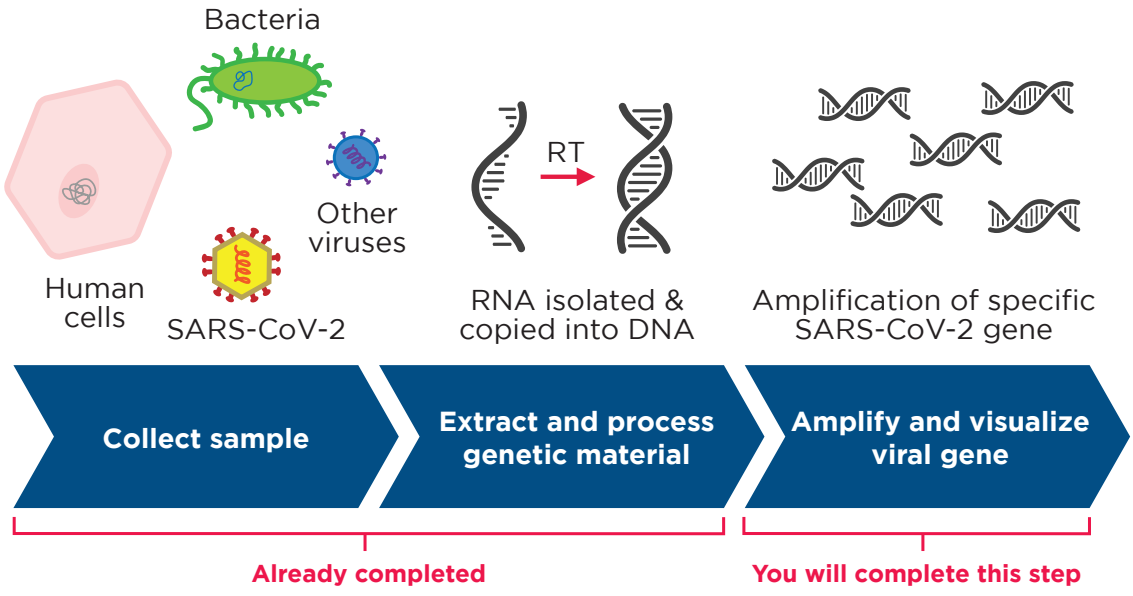


Figure 3. SARS-CoV-2 nucleic acid detection. (1) A sample is collected from the patient, often by swabbing the nasal passage. This sample will contain cells from the patient, as well as any bacteria and viruses that are present. (2) Because SARS-CoV-2 has an RNA genome, scientists isolate RNA from the patient sample. Next, reverse transcription is used to convert the RNA to DNA. This is necessary because the final step, PCR, only works on DNA. (3) To detect specific SARS-CoV-2 genetic sequences, PCR is used. PCR copies specific genetic sequences, allowing scientists to determine if the SARS-CoV-2 virus was present. Finally, scientists analyze the result of the PCR to see if the SARS-CoV-2 viral genetic sequence was detected.

Quantitative PCR (qPCR)

PCR allows scientists to copy a specific sequence of DNA, known as the target sequence. During PCR, the number of copies of the target DNA sequence doubles with each cycle, leading to exponential amplification (Figure 4).

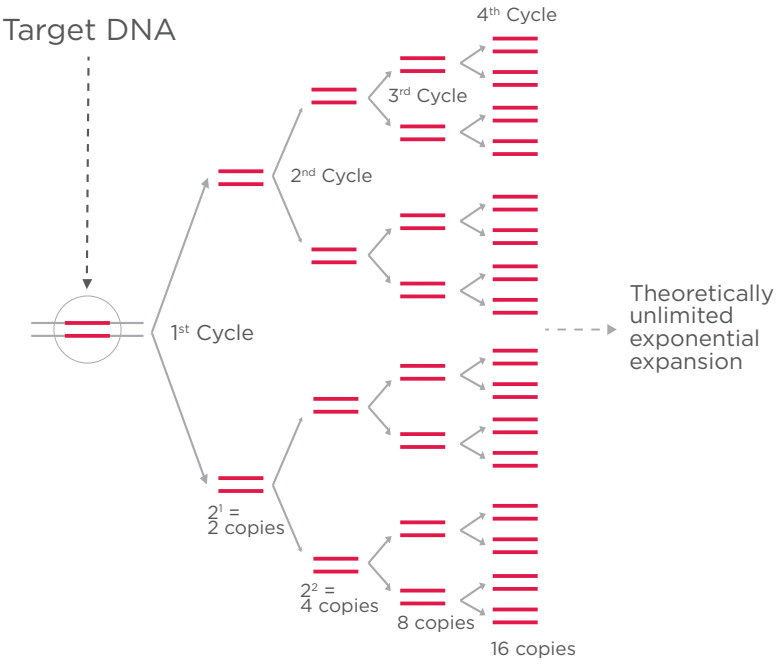


Figure 4. Exponential amplification using PCR. During PCR, the number of copies of the target DNA doubles with each cycle. At the end of a typical PCR run, billions of copies of the specific target have been generated. While the input for PCR during a COVID test is a complex DNA sample with patient DNA, as well genetic material from any bacteria or viruses that were present, PCR allows scientists to copy just the genetic sequence they are interested in. In a COVID PCR test, scientists amplify a SARS-CoV-2 specific gene. If the PCR copies the viral gene, it tells you that the SARS-Cov-2 virus was present, and the patient is diagnosed as being infected.

The ability to copy a specific genetic sequence—and only that sequence—is essential in a COVID test because the sample will contain a complex mix of genetic material from the patient's own cells as well as any bacteria or viruses that were present. In a COVID test, scientists use PCR to copy a gene specific to the SARS-CoV-2 virus. If the PCR copies the viral gene, it tells you that the SARS-CoV-2 virus was present, and the patient is diagnosed as being infected.[2]

Scientists commonly use a variation of PCR called quantitative polymerase chain reaction (qPCR) to diagnose SARS-CoV-2 infection. qPCR copies the DNA and uses a fluorescent readout to simultaneously detect whether or not copies were made.

qPCR is able to track how much of the target sequence is produced in each PCR cycle using a fluorescent dye or probe. This can be done a few different ways, but the simplest is to add a fluorescent dye that will only bind to double-stranded DNA. As the qPCR progresses and makes more and more copies of the target sequence, the tube will fluoresce brighter and brighter. How brightly the reaction fluoresces is a direct readout of how much DNA is in the tube. For some viruses, estimating the amount of viral genetic materiel present in a patient sample, or viral load, can help inform treatment.

When qPCR is performed in a research lab or medical facility, fluorescence is monitored throughout the qPCR by an automated fluorometer—people never actually look at the samples. The qPCR machine produces a graph where the relative fluorescence of each sample is plotted over time (Figure 5), and scientists use these graphs to interpret the qPCR results.

[2]A typical COVID-19 PCR assay tests for two SARS-CoV-2 genes at the same time.

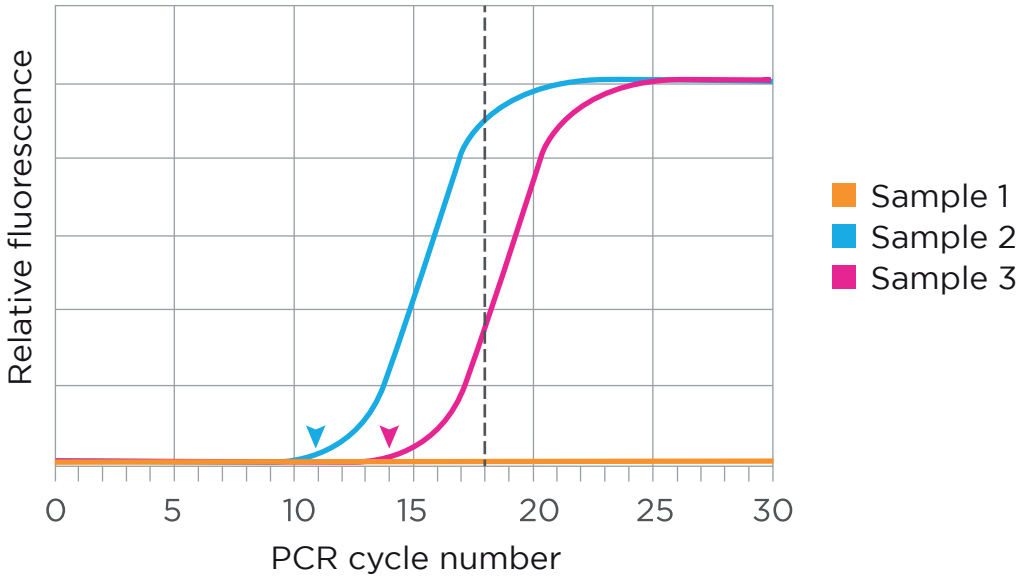


Figure 5. qPCR monitors PCR products over time using fluorescence. During qPCR, a fluorescent dye or probe is used to track the accumulation of PCR products. As copies of the target DNA double with each PCR cycle, fluorescence levels increase and eventually cross a threshold where they are detectable. The more copies of the target sequence that are present in the sample at the start of the PCR, the sooner the threshold will be crossed. In the graph above, Sample 2 had the most copies of the target sequence since the fluorescence for Sample 2 became observable the soonest, after 11 cycles (blue arrowhead). On the other hand, the florescence in Sample 3 became detectable at cycle 14 (pink arrowhead). Comparing two samples at the same time point allows relative quantification. For example, comparing Samples 2 and 3 at cycle 18 (dashed line) also shows that Sample 2 has more product than Sample 3. In this experiment, Sample 1 did not contain the target sequence as evidenced by the lack of florescence.

While there are multiple options for diagnosing SARS-CoV-2 viral infections, qPCR is considered the gold standard for several reasons. First, PCR-based assays are extremely sensitive and can detect infections where relatively few copies of the virus are present. Second, qPCR can be streamlined and automated to reduce the chance of human error or contamination. In many cases, the entire process is automated and can take place in a single tube. The qPCR machine monitors the fluorescence throughout the reaction, and algorithms are applied to determine whether each sample should be categorized as infected or uninfected.

PCR vs. qPCR

PCR is one of the most common and powerful techniques in a molecular biology laboratory. PCR gives scientists the ability to find a specific sequence of DNA in a complex sample and make billions of copies of that specific sequence. But while PCR is an incredibly powerful tool, it is typically an endpoint reaction; this means that once we start a PCR, we don't look at it again until the reaction is over. Quantative PCR (qPCR) puts a spin on this process by monitoring the reaction in real time, using fluorescence to label the copies of DNA as they are produced. During qPCR the amount of fluorescence that is measured is proportional to the amount of DNA being produced—the brighter it glows, the more DNA is present. By tracking the rate of the reaction and comparing that rate to other reactions, it is pos sible to calculate relatively how much of the very specific DNA sequence you started with. For more information, refer to the extension on page 60.

Its ability to not only detect, but also quantify, a genetic sequence of interest has made qPCR a powerful tool in medical diagnostics. By using qPCR, a physician can tell not only if a particular DNA sequence is present, but they can also tell whether it is abundant or rare. For example, a physician may be interested in whether a virus is present in the body, as well as the patient's viral load. qPCR can answer both questions in a single test. Because of this, qPCR is routinely used to diagnose infections with SARS-CoV-2, HIV, hepatitis, and many other viral pathogens.

qPCR is a powerful tool for diagnosing viral infections when you have access to a qPCR machine in a laboratory. However, sometimes it is necessary to deploy testing directly at the point of care. qPCR machines are not well-suited for use outside of a laboratory because they are large (think the size of a microwave or larger), delicate, and expensive, typically costing tens of thousands of dollars.

To address these issues, scientists have developed SARS-CoV-2 nucleic acid detection tests that utilize the same principles as qPCR, but that can be performed without the use of a qPCR machine. Instead of using a specialized qPCR machine, you can use a standard PCR machine and remove the samples to judge the amount of fluorescence visually. Portable and affordable PCR machines like the miniPCR® thermocycler are being deployed for small-scale SARS-CoV-2 nucleic acid detection testing. While this method doesn't have the quantitative precision of an integrated qPCR machine, it allows diagnosticians to accurately determine if a patient is infected with SARS-CoV-2, pretty much anywhere.

Diagnostic tests
It's important to remember that no diagnostic test is perfect, and even highly accurate tests have their limits. For example, when testing for a virus, two important factors are how much virus was present in the patient sample and the sensitivity of the diagnostic test.

The amount of SARS-CoV-2 virus present in a patient sample often depends on how long a person has been infected. For example, if someone has been infected with the SARS-CoV-2 virus very recently, there won't be very much virus present because the virus hasn't had enough time to spread and replicate. For this reason, it's often recommended to wait three to five days after a possible exposure to be tested for COVID-19.

The sensitivity of the diagnostic test depends on the type of test being used. Different testing technologies have diferent detection limits, but qPCR is one of the most sensitive. When you get a COVID-19 test, after they swab your nose, the swab is placed in a vial full of storage buffer. A qPCR test is able to detect as little as 100 copies of viral genetic material per milliliter of storage buffer. To get a sense of how sensitive a COVID qPCR test is, it helps to know that patient swabs that yield positive qPCR COVID-19 tests usually have thousands to billions of copies of viral genetic material per milliliter of storage buffer.

Diagnosticians refer to the lower bound of the sensitivity of the diagnostic test as the limit of detection. If you are infected with a very small amount of virus that is below the limit of detection, it is possible to get a negative test result even though you are actually infected. On the other hand, if the virus has progressed enough to where it is widespread and in high numbers, the test is expected to always return a positive result.

Understanding both the time from infection and the test's sensitivity helps a diagnostician accurately interpret results. But some cases, where the sample sits right at the limit of detection, may yield unclear test results. In these cases, test results will be reported as indeterminate, or inconclusive, and the test will likely need to be repeated. Being clear about what diagnostic tests can and cannot detect, and accurately reporting indeterminate or inconclusive results, can help everyone better interpret test results.

Today's lab

The speed and availability of international travel make it much easier for viral infections to spread globally and become full-blown pandemics. One way to contain viral infections is to test travelers when they enter a country so they can quarantine if they arrive infected. Because symptoms for many diseases are similar, it is not always easy to untangle whether a patient has a run-of-the-mill cold, seasonal allergies, or a dangerous virus. Further, with many viruses, including SARS-CoV-2, a patient may be contagious before they show symptoms, or may even be asymptomatic for the entirety of their infection.

You work at a pop-up screening facility at a small international airport where all incoming patients are tested for SARS-CoV-2. Your latest incoming flight includes a family in which one member developed COVID-like symptoms during their 12-hour international flight. You will use molecular techniques to determine whether anyone in the family is infected with the SARS-CoV-2 virus.

You will test for SARS-CoV-2 infection by using PCR to amplify a ~400 base-pair portion of a SARS-CoV-2 gene that encodes one of the proteins that makes up the viral coat. The patient samples you will be given were prepared as follows. A technician first took a nasal swab to collect a sample from the patient's airways. RNA was extracted from the sample, and reverse transcription was used to convert RNA to DNA (Figure 3). You will perform the last step of the workflow and perform PCR with a fluorescent dye to determine if SARS-CoV-2 genetic material is present in any of the patient samples.

In addition, you will test two important control samples. The first is referred to as a positive control. A *positive control* is a sample where you expect to get a positive result and allows you to assess the validity of your test. You will use a synthetic segment of the viral gene that the PCR is designed to detect. If your PCR does not detect SARS-CoV-2 in the positive control sample, you know something is wrong with the test itself and that the test is invalid.

The second control you will include is referred to as a negative control. A *negative control* is a sample where you expect to get a negative result and allows you to test for contamination. Because PCR is very sensitive and can detect small amounts of viral genetic material, even trace amounts of viral genetic material that are accidentally introduced into a patient's sample will be detected. This could happen if your lab tools became contaminated from a previous positive test. In this case, even though the patient is actually uninfected, their test result will suggest the presence of viral genetic material. Including a negative control sample that is known to lack viral genetic material can help scientists identify when contamination has occurred and the patient tests need to be repeated. You will use ultra-pure water that does not contain any DNA or RNA as a negative control. If your PCR detects SARS-CoV-2 in the negative control sample, you know that your samples may be contaminated.

To determine whether any of your patients are infected with the SARS-CoV-2 virus, you will either:

- Collect endpoint data: If your teacher instructs you to follow this protocol, you will compare the relative fluorescence levels between your four patient samples and your two control samples once the PCR is complete.

OR

- Collect qPCR time point data: If your teacher instructs you to follow this protocol, you will measure the relative fluorescence levels between your four patient samples and your two control samples at several designated times during the PCR.

Both methods will allow you to diagnose your patients for infection with the SARS-CoV-2 virus. The second method has the added benefit that you will be able to compare the relative viral loads between samples, but it requires more time.

Patient descriptions

Your patients are members of the same household and have just returned from a three-week international trip.

Patient AH is a 45-year-old male. He presents with a sore throat and a runny nose, but no fever. His symptoms began shortly after takeoff on a 12-hour international flight. Patient AH is generally in good health.

Patient BH is a 45-year-old female. She has no symptoms. Patient BH is generally in good health.

Patient CH is a 10-year-old female. She has no symptoms. Patient CH is in good health. Patients CH and DH are twins.

Patient DH is a 10-year-old male. He has no symptoms. Patient DH is in good health. Patients CH and DH are twins.

Laboratory guides

Note: The data for this lab can be collected two ways: endpoint observation or qPCR time point observations.

Laboratory guide 1: Endpoint observation

Laboratory guide 2: qPCR time point observations

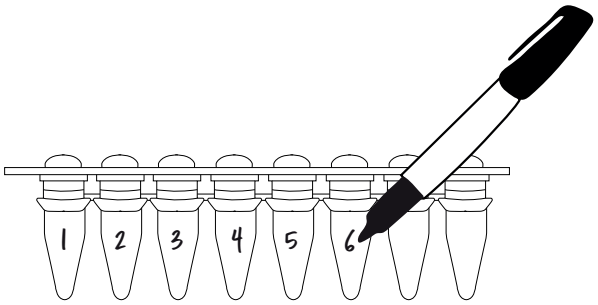
Laboratory guide 3: Optional gel electrophoresis

Laboratory guide 1: Protocol for endpoint observation

Protective gloves and eyewear should be worn for the entirety of this experiment.

PCR setup

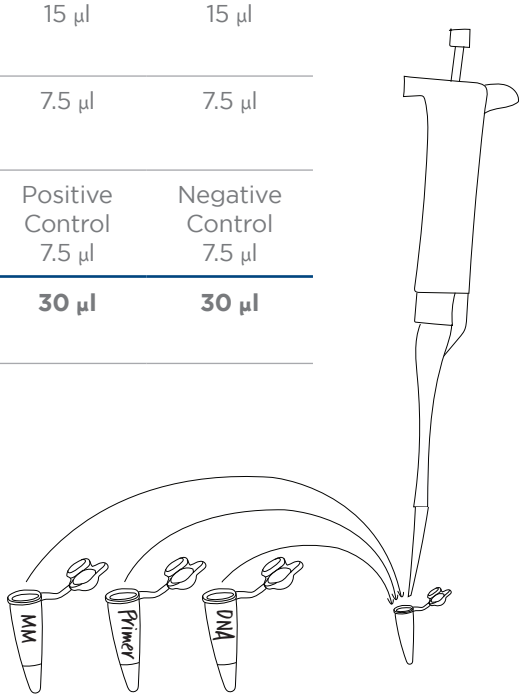
1. **Label 6 PCR tubes (200 µl tubes)**
   - You should receive a strip of 8 tubes, but you will only need 6 of them.
   - Label each tube on the upper portion of the sidewall of the tube.
   - Note: Writing on the cap of the tube or the lower half of the sidewall is likely to rub off later.



2. **Add PCR reagents to each tube**

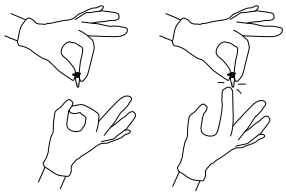
	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
2X qGRN Master Mix	15 µl	15 µl	15 µl	15 µl	15 µl	15 µl
COVID Lab Primers	7.5 µl	7.5 µl	7.5 µl	7.5 µl	7.5 µl	7.5 µl
DNA sample	Patient AH 7.5 µl	Patient BH 7.5 µl	Patient CH 7.5 µl	Patient DH 7.5 µl	Positive Control 7.5 µl	Negative Control 7.5 µl
TOTAL VOLUME	**30 µl**	**30 µl**	**30 µl**	**30 µl**	**30 µl**	**30 µl**

Note: 2X qGRN Master Mix™ contains
- Taq DNA polymerase
- PCR buffer with Mg^{2+}
- dNTPs
- DNA-binding fluorescent dye

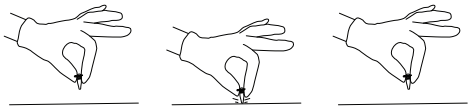


Use a micropipette to add each of the reagents. Remember to change tips at each step!

Flick to mix



Tap to collect liquid at bottom



3. **Cap the tubes and ensure the reagents mix well**
    - Flick each tube to ensure proper mixing.
    - Gently tap the tubes on your bench to ensure that the liquid collects at the bottom.

4. **Place the tubes inside the miniPCR® machine**
    - Press firmly on the tube caps to ensure a tight fit.
    - Close the PCR machine lid and tighten it gently.

PCR programming

These instructions are illustrated using miniPCR® software on a Windows PC. Software interfaces vary slightly by operating system. See the miniPCR® User's Guide for more details.

If using a different thermal cycler, PCR protocol parameters should remain the same (step 7).

1. **Open the miniPCR® app and remain on the "Library" window**

2. **Connect your miniPCR® thermal cycler to your device using the supplied USB cable or via Bluetooth®**
   - Note: Bluetooth® is only available on certain models. To connect via Bluetooth®, select the (m) icon, located by "Devices" at the left of the desktop app or at the top of the mobile app.

3. **Make sure your miniPCR® thermal cycler is plugged in and that the power switch is turned on**
   - Note: If your machine begins running a previously loaded protocol, you may stop it by clicking or tapping the "X" symbol in the top left box of the "Monitor" window.

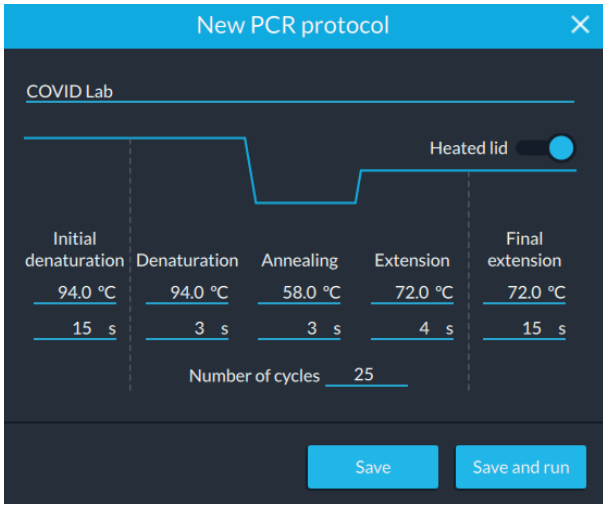
4. **While in the "Library" window, click the ⊕ button to create a new protocol**
   - Button is located in the upper right hand corner of the window.

5. **Select "PCR" from the drop-down menu**

6. **Enter a name for the protocol; for example: "COVID Lab"**

7. **Enter the PCR protocol parameters:**
   - Initial denaturation 94°C, 15 sec
   - Denaturation 94°C, 3 sec
   - Annealing 58°C, 3 sec
   - Extension 72°C, 4 sec
   - Number of cycles 25
   - Final extension 72°C, 15 sec

   Note: The "Heated lid" slider should be in the on position.



8. **Click "Save and run" to start the protocol**
   - If connected to more than one machine, choose the serial number of the miniPCR® thermal cycler you are using. If asked "Do you want to stop the current protocol…?", click "Yes".
   - The lights on the front of the miniPCR® thermal cycler will blink 3 times to indicate that the protocol has been loaded.
   - Note: If needed, you may unplug the USB cable or disconnect Bluetooth® once the protocol has been loaded. Even if disconnected from your device, the protocol will continue to completion as normal.

9. **Choose "Monitor" window**
   - The "Monitor" window can be selected on the left column in the desktop app and at the top in mobile app.
   - If more than one miniPCR® thermal cycler is connected to the same device, choose which machine you would like to monitor using the tabs at the top of the window (desktop app) or bottom of the Library (mobile app).

The miniPCR® software allows each lab group to monitor the reaction parameters in real time.

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10. **To collect data, observe your samples at 72°C**
    - You can either:
      a. Monitor the PCR reactions and pause the program during the final extension and observe fluorescence.

    OR

      b. Wait until the PCR run has completed (approximately 35 min) then heat the samples to 72°C and observe fluorescence.
    - Detailed instructions are provided on the pages 26-27.

Data collection

Important note: All samples, including the negative control, will be fluorescent at room temperature. Samples must be observed at 72°C.

- Room temperature is cool enough that the primers used to amplify the DNA will bind to each other to create double-stranded DNA even though they are not complementary. The DNA dye will bind to these primer duplexes and emit fluorescence.
- You must heat your samples to 72°C before data collection. At this temperature, the primers in solution will be single stranded, while the newly synthesized DNA will remain double stranded.

Data collection during final extension

1. **Press the Pause ⏸ button as soon as the machine enters the final extension stage**
   - The machine will heat the samples to 72°C and then hold at that temperature.
   - Allow your samples to remain at 72°C for at least 10 seconds.

   - Pressing pause before miniPCR® reaches 72°C is OK. If the machine has entered the extension phase, but has not yet reached 72°C, it will continue heating until temperature is reached and will pause at that time.
   - If you press pause too late in the cycle, the program will end. In this case, heat the samples to 72°C using a miniPCR® in heat block mode or any other heat block.

2. **Proceed to step 3 on the next page**

Data collection after completion of PCR

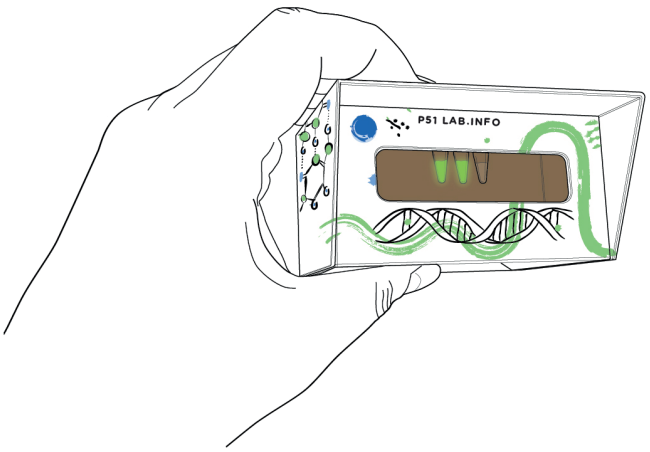
1. **Let the PCR program run to completion (approximately 35 minutes)**
   - The app status will show "Finished" and the red, yellow, and green LEDs on your miniPCR® thermal cycler will light up and stay on.
   - PCR product is stable at room temperature for several days. For longer term storage, move tubes to a fridge or freezer.

2. **Heat samples for 1 minute at 72°C, then proceed to step 3**
   - Use a miniPCR® in heat block mode or any other heat block.

3. **Open the miniPCR® machine and remove your samples**

! Be careful not to touch the metal lid which may still be hot

4. **<u>Quickly</u> place the tubes in the P51™ molecular fluorescence viewer or other blue light illuminator**
   - <u>Immediately</u> record your observations in the data table (Page 33).
   - Record your data using the following semi-quantitative scale:
     1- no fluorescence / 1- dim fluorescence / 2- moderate fluorescence / 3- full fluorescence
   - Use the positive and negative controls as a reference:
     - The positive control is expected to be 3- full fluorescence
     - The negative control is expected to be 0- no fluorescence
   - Remember that as the samples cool, the primers can bind non-specifically and all the samples will fluoresce. If the negative control begins to fluoresce, just heat the samples again for 10 seconds at 72°C before trying to record your results.



Pre-lab study questions

Review

1. **What is the difference between SARS-CoV-2 and COVID-19?**

2. **What are the two main parts of a virus?**

3. **Which part of a virus does qPCR detect?**

4. **In diagnosing a patient with a viral infection, why is it better to use a nucleic acid detection test than to make a diagnosis based only on the patient's symptoms?**

5. **Explain why a reverse transcription step needs to be performed before using qPCR to detect SARS-CoV-2 infection.**

6. **List two reasons why qPCR is considered the gold standard for detecting SARS-CoV-2 infection.**

7. **Differentiate between a positive control and a negative control.**

Critical thinking

1. **According to the Centers for Disease Control and Prevention (CDC): People with COVID-19 have had a wide range of symptoms reported—ranging from mild symptoms to severe illness. Symptoms may appear 2-14 days after exposure to the virus. Anyone can have mild to severe symptoms.**
   People with these symptoms may have COVID-19:

   - Fever or chills
   - Cough
   - Shortness of breath or difficulty breathing
   - Fatigue
   - Muscle or body aches
   - Headache
   - New loss of taste or smell
   - Sore throat
   - Congestion or runny nose
   - Nausea or vomiting
   - Diarrhea

   Based on this information, do you think any of your patients are infected with SARS-CoV-2? How confident are you in your diagnoses?

2. **You will carry out a nucleic acid detection test on samples from each of your patients. The samples will contain genetic material from different sources: from the patients' own cells, from bacteria present in the nasal passage, and potentially from viruses, too. Explain how you can be sure that you are testing specifically for SARS-CoV-2.**

3. **qPCR combines PCR and fluorescence detection. Summarize how fluorescence can be used to determine if a sample contains genetic material from the SARS-CoV-2 virus.**

4. **Recall that the brightness of the fluorescence in this PCR test is proportional to the amount of viral genetic material in the patient's sample. Imagine one patient's sample is fluorescent at the end of the PCR test, but not as brightly fluorescent as the positive control sample. How would you diagnose this patient? Explain your reasoning.**

5. **In diagnostic testing, there are two possible types of incorrect test results. A** false negative **is a result that incorrectly indicates an absence of infection, whereas a** false positive **is a result that incorrectly indicates the presence of an infection.**

   a. **In your opinion, is it more dangerous to have a false negative or a false positive result when testing for SARS-CoV-2 infection? In which case are the potential consequences worse? Justify your answer.**

   b. **Which experimental control that you will test can guard against a false negative test result? Explain your reasoning.**

c. **Which experimental control that you will test can guard against a false positive test result? Explain your reasoning.**

d. **Another control typically performed when testing for viral infection is to amplify a human gene that should be present in all patient samples since we inevitably collect some of the patients' own cells along with the viral material. Does this control guard against false negative or false positive results? Explain your reasoning.**

Post-lab study questions for endpoint observation

Interpreting results

1. **Record your data using the following semi-quantitative scale:**
   0- no fluorescence / 1- dim fluorescence / 2- moderate fluorescence / 3- full fluorescence
   Use the positive and negative controls as a reference:
   - **The positive control is expected to be 3- full fluorescence**
   - **The negative control is expected to be 0- no fluorescence**

Patient AH	Patient BH	Patient CH	Patient DH	Positive Control	Negative Control

2. **After reviewing the results of the experiment, how would you diagnose your patients? Explain your reasoning.**

Patient AH:

Patient BH:

Patient CH:

Patient DH:

3. **How certain are you of your diagnoses? Explain your reasoning.**

Critical thinking

4. **What measures do you think the members of this family should take in light of their diagnoses? Explain your reasoning.**

CER Table

Fill in the table based on your results from the lab. Use the rubric on the next page to guide your answers.

Question:

Are any of your patients infected with SARS-CoV-2?

Claim

Make a clear statement that answers the above question.

Evidence

Provide data from the lab that supports your claim.

Reasoning

Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.

Score	4	3	2	1
CLAIM A statement that answers the original question/ problem.	Makes a clear, accurate, and complete claim.	Makes an accurate and complete claim.	Makes an accurate but incomplete or vague claim.	Makes a claim that is inaccurate.
EVIDENCE Data from the experiment that supports the claim. Data must be relevant and sufficient to support the claim.	All of the evidence presented is highly relevant and clearly sufficient to support the claim.	Provides evidence that is relevant and sufficient to support the claim.	Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence.	Only provides evidence that does not support claim.
REASONING Explain why your evidence supports your claim. This must include scientific principles/ knowledge that you have about the topic to show why the data counts as evidence.	Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning.	Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed.	Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly.	Provides reasoning that does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly.

We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

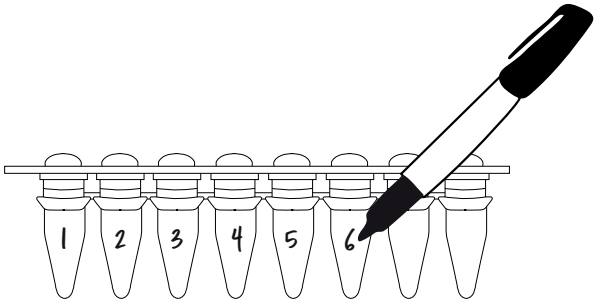
Rubric score	3	4	5	6	7	8	9	10	11	12
Equivalent Grade	55	60	65	70	75	80	85	90	95	100

Laboratory guide 2: Protocol for qPCR time point observations

Protective gloves and eyewear should be worn for the entirety of this experiment.

PCR setup

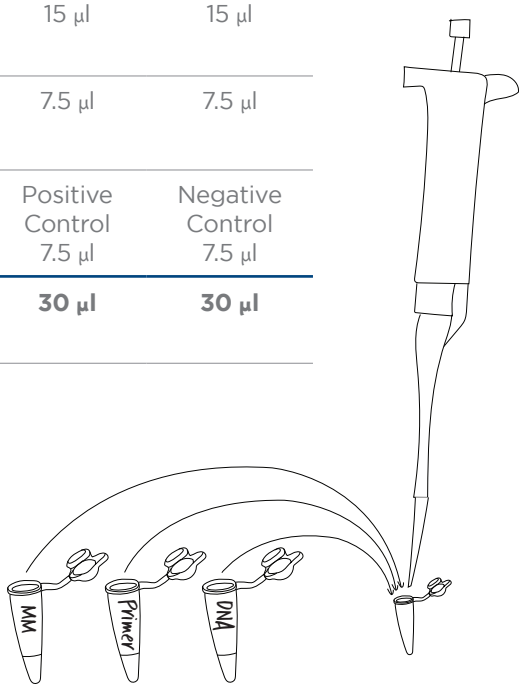
1. **Label 6 PCR tubes (200 µl tubes)**
   - You should receive a strip of 8 tubes, but you will only need 6 of them.
   - Label each tube on the upper portion of the sidewall of the tube.
   - Note: Writing on the cap of the tube or the lower half of the sidewall is likely to rub off later.



2. **Add PCR reagents to each tube**

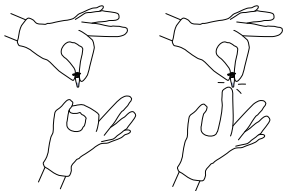
	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
2X qGRN Master Mix	15 µl	15 µl	15 µl	15 µl	15 µl	15 µl
COVID Lab Primers	7.5 µl	7.5 µl	7.5 µl	7.5 µl	7.5 µl	7.5 µl
DNA sample	Patient AH 7.5 µl	Patient BH 7.5 µl	Patient CH 7.5 µl	Patient DH 7.5 µl	Positive Control 7.5 µl	Negative Control 7.5 µl
TOTAL VOLUME	**30 µl**	**30 µl**	**30 µl**	**30 µl**	**30 µl**	**30 µl**

Note: 2X qGRN Master Mix™ contains:
• Taq DNA polymerase • dNTPs
• PCR buffer with Mg^{2+} • DNA-binding fluorescent dye

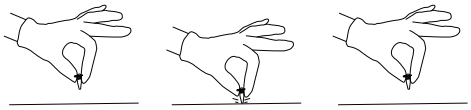


Use a micropipette to add each of the reagents. Remember to change tips at each step!

Flick to mix



Tap to collect liquid at bottom



3. **Cap the tubes and ensure the reagents mix well**
    - Flick each tube to ensure proper mixing.
    - Gently tap the tubes on your bench to ensure that the liquid collects at the bottom.

4. **Place the tubes inside the miniPCR® machine**
    - Press firmly on the tube caps to ensure a tight fit.
    - Close the PCR machine lid and tighten it gently.

PCR programming

These instructions are illustrated using miniPCR® software on a Windows PC. Software interfaces vary slightly by operating system. See the miniPCR® User's Guide for more details.

If using a different thermal cycler, PCR protocol parameters should remain the same (step 7).

1. **Open the miniPCR® app and remain on the "Library" window**

2. **Connect your miniPCR® thermal cycler to your device using the supplied USB cable or via Bluetooth®**
   - Note: Bluetooth® is only available on certain models. To connect via Bluetooth®, select the (m) icon, located by "Devices" at the left of the desktop app or at the top of the mobile app.

3. **Make sure your miniPCR® thermal cycler is plugged in and that the power switch is turned on**
   - Note: If your machine begins running a previously loaded protocol, you may stop it by clicking or tapping the "X" symbol in the top left box of the "Monitor" window.

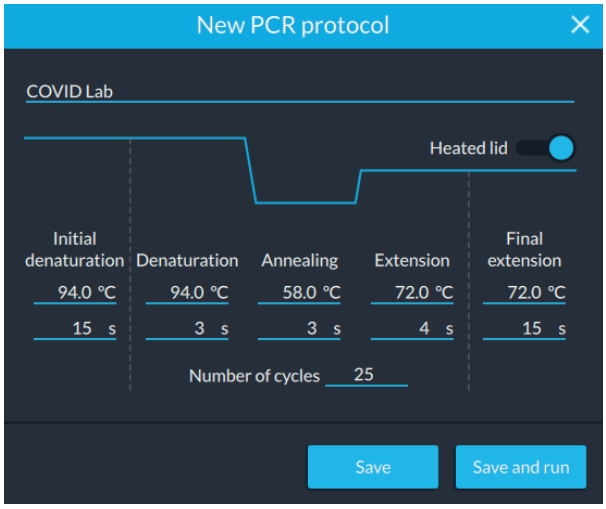
4. **While in the "Library" window, click the ⊕ button to create a new protocol**
   - Button is located in the upper right hand corner of the window.

5. **Select "PCR" from the drop-down menu**

6. **Enter a name for the protocol; for example: "COVID Lab"**

7. **Enter the PCR protocol parameters:**
   - Initial denaturation 94°C, 15 sec
   - Denaturation 94°C, 3 sec
   - Annealing 57°C, 3 sec
   - Extension 72°C, 4 sec
   - Number of cycles 25
   - Final extension 72°C, 15 sec

   Note: The "Heated lid" slider should be in the on position.



Instructions for collecting data throughout the PCR

Important note: All samples, including the negative control, will be fluorescent at room temperature. Samples must be observed at 72°C.

- Room temperature is cool enough that the primers used to amplify the DNA will bind to each other to create double-stranded DNA even though they are not complementary. The DNA dye will bind to these primer duplexes and emit fluorescence.
- You must heat your samples to 72°C before data collection. At this temperature, the primers in solution will be single stranded, while the newly synthesized DNA will remain double stranded.
- You will pause the PCR program during the "extension" step of a PCR cycle when samples are at 72°C.
- Each step in the PCR cycle (denaturation, annealing, extension) is less than 5 seconds long, so you need to carefully monitor the reaction to make sure you pause the machine and collect data at the correct time.
- You will collect data during cycle 1, then every three cycles starting with cycle 10.

1. **Click "Save and run" to start the protocol**
   - If asked "Do you want to stop the current protocol...?", click "Yes".
   - The lights on the front of the miniPCR® thermal cycler will blink 3 times to indicate that the protocol has been loaded.

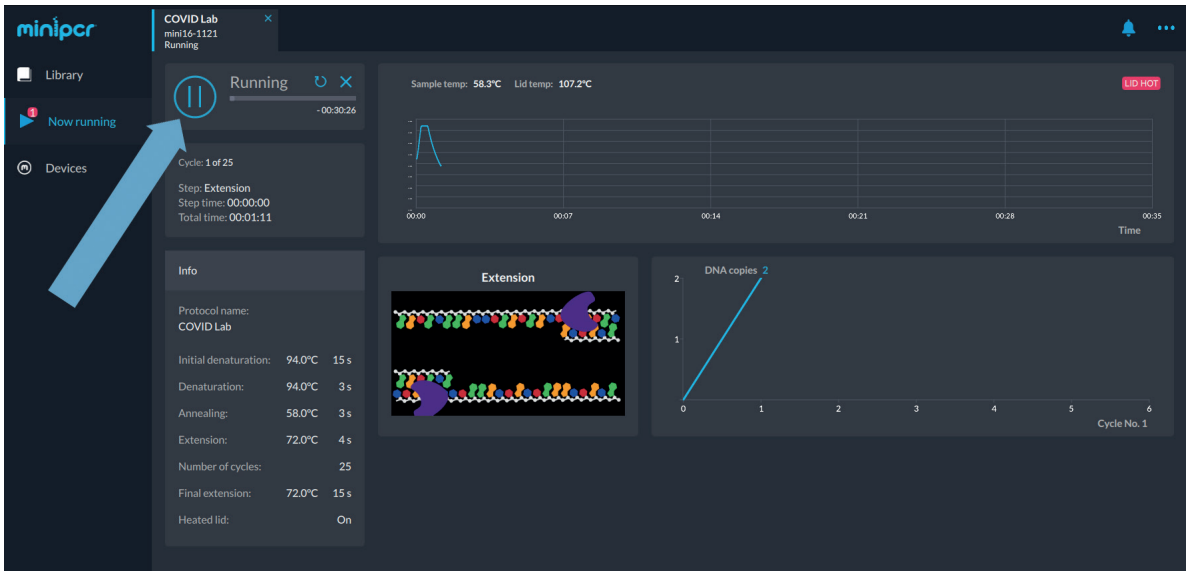
2. **Choose "Monitor" window**
   - The "Monitor" window can be selected on the left column in the desktop app and at the top in mobile app.

Continued on the next page

Initial observation at cycle 1

3. **Press the Pause ⏸ button <u>as soon as</u> the machine enters the extension stage**
    - The machine will heat the samples to 72°C and then hold at that temperature.
    - Allow your samples to remain at 72°C for at least 5 seconds before removing.
    - Open the miniPCR® machine and remove your samples.

Be careful not to touch the metal lid which may still be hot

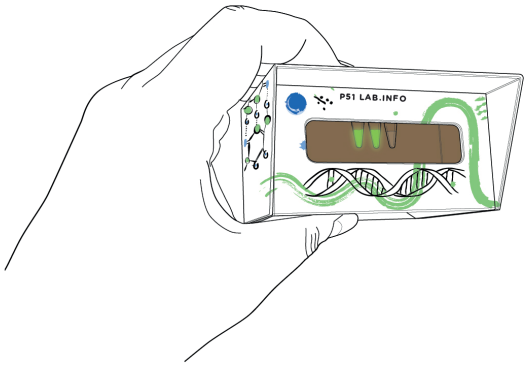


- Pressing pause before miniPCR® reaches 72°C is OK. If the machine has entered the extension phase, but has not yet reached 72°C, it will continue heating until temperature is reached and will pause at that time.

- If you press pause too late in the cycle, the machine will continue on to the denaturation stage and hold at 94°C. If this happens, your samples will denature and no fluorescence will be visible. Skip this cycle and measure at the next extension step.

Continued on the next page

4. **Quickly place the tubes in the P51™ molecular fluorescence viewer or other blue light illuminator**
    - Immediately record your observations on the 'cycle 1' row of the data table (Page 44).
    - Record your data using the following semi-quantitative scale:
      0- no fluorescence
      1- dim fluorescence
      2- moderate fluorescence
      3- full fluorescence
    - Remember that as the samples cool, the primers can bind non-specifically and all the samples will fluoresce. If this happens, just put the samples back in the PCR machine at 72°C for 10 seconds before trying to record your results.



5. **Return the tubes to the miniPCR® machine and press the Run ▶ button**

Subsequent observations starting at cycle 10

6. **Allow the PCR to continue until cycle 10**
    - It will take approximately 10 minutes to reach cycle 10 of your PCR protocol.
    - Keep an eye on the progress of your PCR. You don't want to miss your observation time point!

Continued on the next page

7. **Once the PCR reaches cycle 10, press the Pause ⏸ button <u>as soon as</u> the machine enters the extension stage**
    - The machine will heat the samples to 72°C and then hold at that temperature.
    - Allow your samples to remain at 72°C for at least 5 seconds before removing.
    - Open the miniPCR® machine and remove your samples.

Be careful not to touch the metal lid which may still be hot

8. **<u>Quickly</u> place the tubes in the P51™ molecular fluorescence viewer or other blue light illuminator**
    - <u>Immediately</u> record your observations on the 'cycle 10' row of the data table on the next page.

9. **Return the tubes to the miniPCR® machine and press the Run ▶ button**

10. **Repeat steps 7-9 to collect data according to the chart below:**

Fluorescence level at 72˚C						
Cycle #	Patient AH	Patient BH	Patient CH	Patient DH	Positive Control	Negative Control
1						
10						
13						
16						
19						
22						
25						

- Record your data using the following semi-quantitative scale:
  0- no fluorescence / 1- dim fluorescence / 2- moderate fluorescence / 3- full fluorescence
- Remember that as the samples cool, the primers can bind non-specifically and all the samples will fluoresce. If this happens, just put the samples back in the PCR machine at 72°C for 10 seconds before trying to record your results.

11. **When the PCR run has completed, app status will show "Finished" and the red, yellow, and green LEDs on your miniPCR® thermal cycler will light up and stay on**

! Be careful not to touch the metal lid which may still be hot

12. **PCR product is stable at room temperature for several days. For longer term storage, move tubes to a fridge or freezer**
    - Tubes may remain inside the miniPCR® thermal cycler for several days following protocol completion.

Pre-lab study questions

Review

1. **What is the difference between SARS-CoV-2 and COVID-19?**

2. **What are the two main parts of a virus?**

3. **Which part of a virus does qPCR detect?**

4. **In diagnosing a patient with a viral infection, why is it better to use a nucleic acid detection test than to make a diagnosis based only on the patient's symptoms?**

5. **Explain why a reverse transcription step needs to be performed before using qPCR to detect SARS-CoV-2 infection.**

6. **List two reasons why qPCR is considered the gold standard for detecting SARS-CoV-2 infection.**

7. **Differentiate between a positive control and a negative control.**

Critical thinking

1. **According to the Centers for Disease Control and Prevention (CDC): People with COVID-19 have had a wide range of symptoms reported—ranging from mild symptoms to severe illness. Symptoms may appear 2-14 days after exposure to the virus. Anyone can have mild to severe symptoms.**
   People with these symptoms may have COVID-19:

   - Fever or chills
   - Cough
   - Shortness of breath or difficulty breathing
   - Fatigue
   - Muscle or body aches
   - Headache
   - New loss of taste or smell
   - Sore throat
   - Congestion or runny nose
   - Nausea or vomiting
   - Diarrhea

   Based on this information, do you think any of your patients are infected with SARS-CoV-2? How confident are you in your diagnoses?

2. **You will carry out a nucleic acid detection test on samples from each of your patients. The samples will contain genetic material from different sources: from the patients' own cells, from bacteria present in the nasal passage, and potentially from viruses, too. Explain how you can be sure that you are testing specifically for SARS-CoV-2.**

3. **qPCR combines PCR and fluorescence detection. Summarize how fluorescence can be used to determine if a sample contains genetic material from the SARS-CoV-2 virus.**

4. **In diagnostic testing, there are two possible types of incorrect test results. A** false negative **is a result that incorrectly indicates an absence of infection, whereas a** false positive **is a result that incorrectly indicates the presence of an infection.**

   a. **In your opinion, is it more dangerous to have a false negative or a false positive result when testing for SARS-CoV-2 infection? In which case are the potential consequences worse? Justify your answer.**

   b. **Which experimental control that you will test can guard against a false negative test result? Explain your reasoning.**

   c. **Which experimental control that you will test can guard against a false positive test result? Explain your reasoning.**

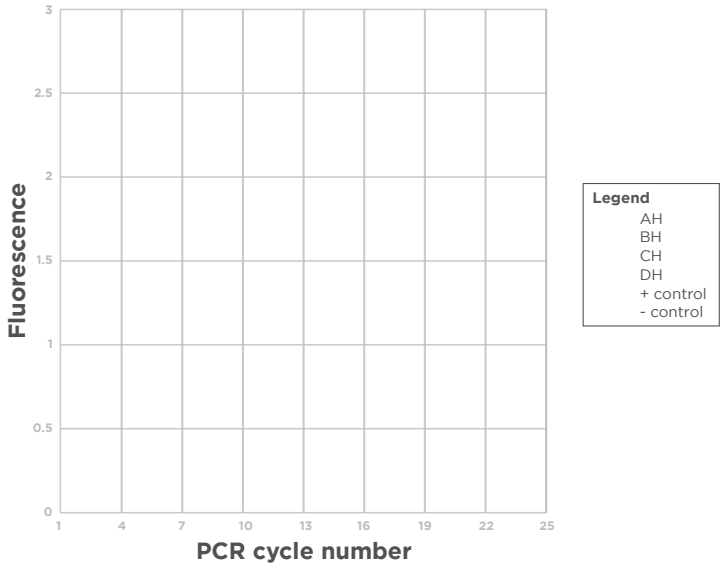
Questions continue on next page.

d. **Another control typically performed when testing for viral infection is to amplify a human gene that should be present in all patient samples since we inevitably collect some of the patients' own cells along with the viral material. Does this control guard against false negative or false positive results? Explain your reasoning.**

Post-lab study questions for qPCR time point observations

Interpreting results

1. **Graph your data. Be sure to make your six data sets look different by using a different color or style of data point each sample (AH, BH, etc.). Indicate which symbols represent which samples in the graph legend.**



2. **After reviewing the results of the experiment, how would you diagnose your patients? Explain your reasoning.**

Patient AH:

Patient BH:

Patient CH:

Patient DH:

3. **Can you draw any conclusions about the relative viral loads in your patients? Explain your reasoning.**

4. **What measures do you think the members of this family should take in light of their diagnoses? Explain your reasoning.**

CER Table

Fill in the table based on your results from the lab. Use the rubric on the next page to guide your answers.

Question:

Are any of your patients infected with SARS-CoV-2?

Claim

Make a clear statement that answers the above question.

Evidence

Provide data from the lab that supports your claim.

Reasoning

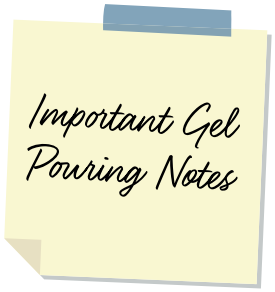
Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.

Score	4	3	2	1
CLAIM A statement that answers the original question/ problem.	Makes a clear, accurate, and complete claim.	Makes an accurate and complete claim.	Makes an accurate but incomplete or vague claim.	Makes a claim that is inaccurate.
EVIDENCE Data from the experiment that supports the claim. Data must be relevant and sufficient to support the claim.	All of the evidence presented is highly relevant and clearly sufficient to support the claim.	Provides evidence that is relevant and sufficient to support the claim.	Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence.	Only provides evidence that does not support claim.
REASONING Explain why your evidence supports your claim. This must include scientific principles/ knowledge that you have about the topic to show why the data counts as evidence.	Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning.	Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed.	Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly.	Provides reasoning that does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly.

We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

Rubric score	3	4	5	6	7	8	9	10	11	12
Equivalent Grade	55	60	65	70	75	80	85	90	95	100

Laboratory Guide 3: Protocol for optional gel electrophoresis



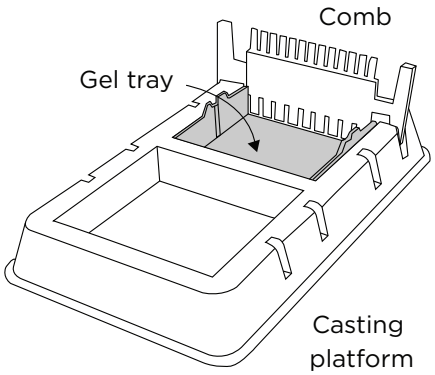
Gels can be prepared up to three days ahead of time and stored at ambient temperature, covered in air-tight plastic wrap and protected from light.

You will need 6 lanes plus one lane for ladder per group. It is possible for groups to share a gel by using two combs.

These instructions are designed for use with the blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.

1. **Prepare 1X TBE buffer (to be completed by teacher in advance)**
   - TBE buffer is often provided as liquid concentrate or powder.
   - Follow manufacturer's instructions to prepare 1X TBE buffer solution.

2. **Prepare a clean and dry casting platform with a gel tray and comb**
   - Place the clear gel tray in the white casting platform.
   - Place a well-forming comb at the top of the gel tray.



3. **Prepare a 2% agarose solution with a fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®) using the method indicated by your instructor**

IMPORTANT NOTE: There are several ways to prepare agarose gels.

- Scan the QR code for detailed instructions on how to prepare agarose gels.
- Both written and video instructions are available.

www.minipcr.com/agarose-gel/

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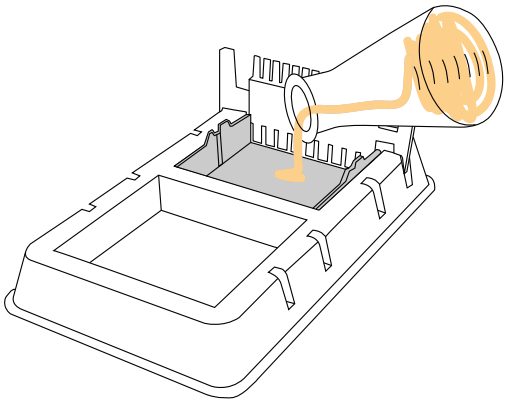
4. **Pour the agarose solution into the prepared casting platform with a gel tray and comb**
    - The agarose solution should cover the bottom of the gel tray and the bottom 3 mm of the comb (roughly the bottom 1/3 of the comb).

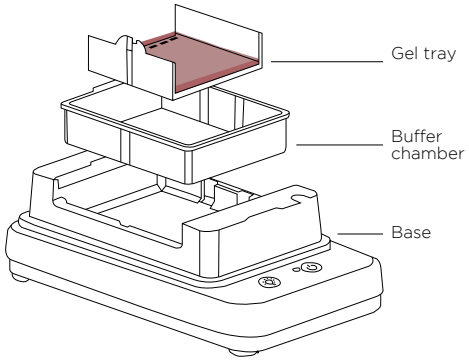
5. **Allow gel to solidify completely and remove the comb by pulling firmly upwards**
    - Gels will typically be ready in about 10 minutes.
    - Gel is ready when cool and firm to the touch.



Gel electrophoresis - Running the gel

These instructions are designed for use with blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.

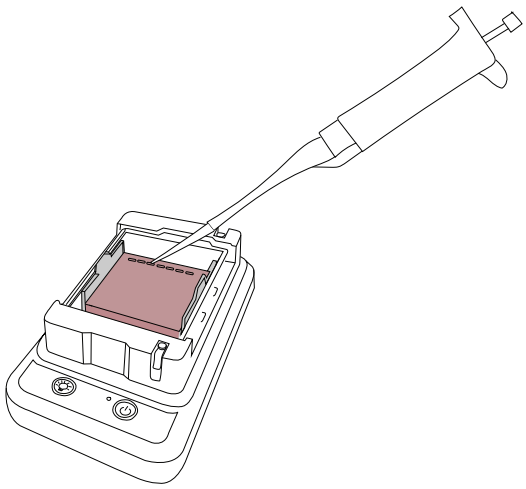
1. **Place the gel tray containing your gel in the buffer chamber**
   - Ensure that the clear buffer chamber is inside the blueGel™ electrophoresis system.
   - The wells of the gel should be on the same side as the negative electrode, away from the power button.



2. **Add 30 ml of 1X TBE electrophoresis buffer**
   - The buffer should just cover the gel and fill the wells.
   - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged).

3. **Add loading dye to your samples**
   - Add 4 µl of loading dye to each tube.
   - Pipette up and down until thoroughly mixed.

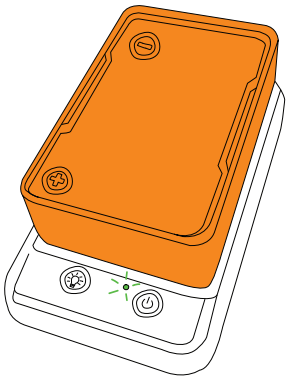
   Note: Change pipette tips between samples to prevent contamination.



4. **Load samples onto the gel in the following sequence**
   - **Lane 1:** 10 µl Fast DNA Ladder 1
   - **Lane 2:** 15 µl Patient AH PCR product
   - **Lane 3:** 15 µl Patient BH PCR product
   - **Lane 4:** 15 µl Patient CH PCR product
   - **Lane 5:** 15 µl Patient DH PCR product
   - **Lane 6:** 15 µl Positive Control PCR product
   - **Lane 7:** 15 µl Negative Control PCR product

   Note: Change pipette tips between samples to prevent contamination.

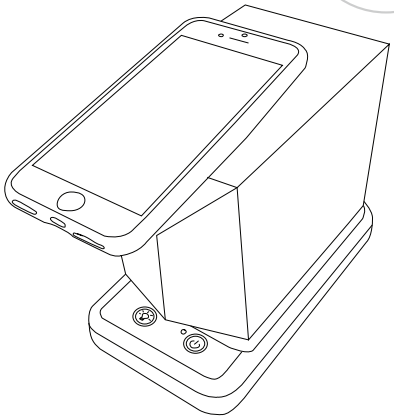
5. **Place the orange cover on the blueGel™ electrophoresis system**
    - To prevent fogging, make sure that ClearView™ spray has been evenly applied to the inside of the orange cover.
    - Match the positive and negative electrode signs on the orange lid with the corresponding positive and negative signs on the blue base.
    - The electrodes of the lid should be aligned with the metal leads on the base.
    - The orange lid should sit flush with the blue base using little force.



6. **Press the "Run" ⏻ button**
    - Check that the green light beside the power button remains illuminated.

7. **Conduct electrophoresis for 15-25 minutes**
    - Note: Check the progress of your samples every 10 minutes to monitor the migration of your DNA samples.
    - Longer electrophoresis times will result in better separation of similar sized DNA fragments. However, if run too long, small DNA fragments can run off the end of the gel or lose fluorescence.

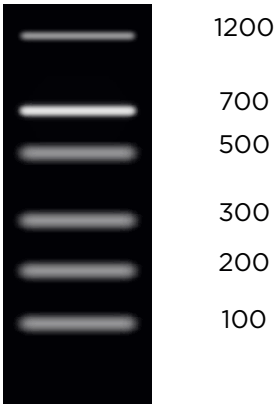
Gel electrophoresis – Visualizing results



1. **Press the "light bulb" button to turn on the blueGel™ transilluminator**
    - For best viewing, dim lights or use Fold-a-View™ photo documentation hood with a smartphone camera.
    - Gels may be viewed at the end of the run or periodically throughout the run.
    - If the image appears hazy, wipe off the inside of the orange cover and reapply ClearView™ spray.

2. **Ensure that there is sufficient DNA band resolution**
    - Run the gel longer if needed to increase resolution.

3. **Document your results**
    - Place Fold-a-View™ photo documentation hood on the blueGel™ electrophoresis system to take a picture with a smartphone or other digital camera.
    - Compare the bands from the DNA samples to the ladder to obtain size estimates.



Post-lab study questions for optional gel electrophoresis

Interpreting results

1. **Use the image on the right to illustrate your gel electrophoresis results. There are seven lanes on the gel: one for your ladder, and one for each sample. Be sure to label each lane with the source of the sample.**

2. **Do the results of your gel confirm the diagnoses you made based on fluorescence? Explain your reasoning.**

3. **What information can you get from your gel that you could not get from observing your reactions in a tube?**

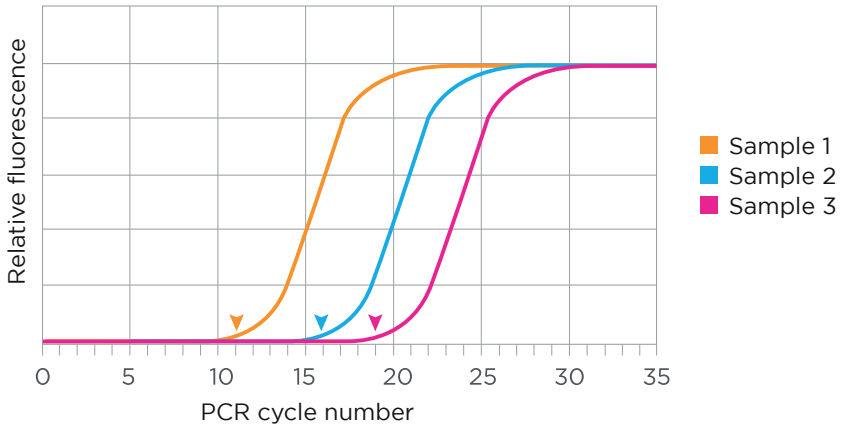
Extension Activities

Extension: Quantification with qPCR

qPCR machines contain a built-in sensor that measures the fluorescence of every sample after each PCR cycle. When you assess the fluorescence after each PCR cycle, you can determine how many cycles it takes for the fluorescence to be detectable. The cycle where fluorescence is first observable above background levels is called Ct, for *cycle threshold.*

How soon a reaction reaches the threshold is directly related to how much of the target sequence was in the sample at the starting point. The more target DNA there is to begin with, the sooner Ct will be reached. This means that by observing Ct, we can learn about our original DNA concentration. Because the amount of target DNA doubles every cycle, if reaction A reaches Ct one cycle earlier than reaction B, you can estimate that reaction A started with roughly twice as much target sequence as reaction B. If reaction A reaches Ct two cycles before reaction C, you can estimate that there was a four-fold difference in the original amount of the target sequence, and so on.

The following graph represents the relative fluorescence of three different samples during a qPCR experiment. Use the information in the graph to answer the questions below.



1. Record the Ct for each sample in the table below:

Sample	Ct
1	
2	
3	

2. **Which sample contained the greatest concentration of the target DNA? Explain your reasoning, and be sure to reference the Ct for the sample in your answer.**

3. **Which sample contained the lowest concentration of the target DNA? Explain your reasoning, and be sure to reference the Ct for the sample in your answer.**

4. **What is the relative difference in the starting concentration of the target DNA between Sample 2 and Sample 3? You may use a calculator, but show your work.**

5. **For some viruses, determining the number of viral particles present in a patient, or viral load, is important in determining the best course of treatment. The typical COVID-19 test is done in a qPCR machine and therefore will report a Ct value for each patient, even though the result is usually just reported as positive or negative. The Ct value corresponds to viral load, and researchers are currently comparing Ct values to patient outcomes for COVID-19 patients.**

   What would be your hypothesis about the relationship between a COVID-19 patient's Ct value and the severity of their disease? Explain your reasoning.

Additional background: COVID-19 testing

There are numerous lab tests that doctors use to diagnose COVID-19, and the testing landscape can change rapidly as new tests are developed or testing guidelines are updated. Refer to the Food and Drug Administration for the most up-to-date information on COVID-19 testing: https://www.fda.gov/consumers/consumer-updates/coronavirus-disease-2019-testing-basics.

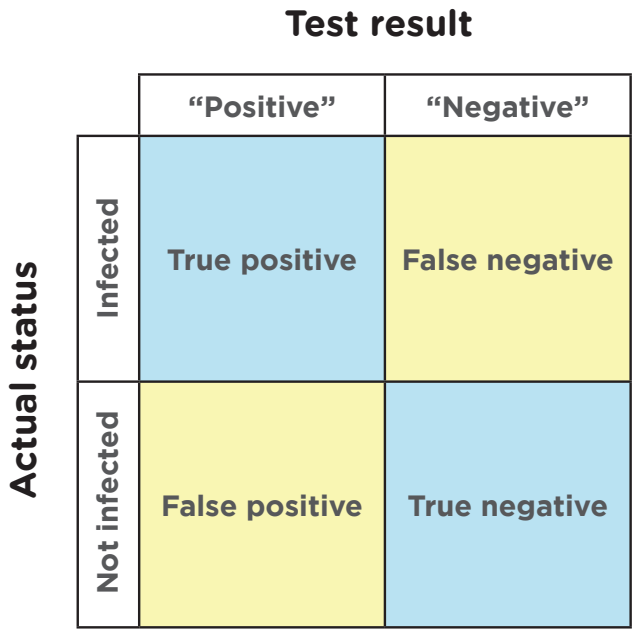
Diagnostic test accuracy

There are two important measures of diagnostic tests: sensitivity and specificity.

Sensitivity is a measure of how well a test can correctly generate a positive test result for people who do have the condition being tested for. Having a test with high sensitivity is important to prevent false negative test results. A *false negative* is a result that incorrectly indicates an absence of the condition being tested for. For example, if someone received a negative COVID test result, when in fact, they were infected with the SARS-CoV-2 virus.

Specificity is a measure of how well a test can correctly generate a negative test result for people who do not have the condition being tested for. Having a test with high specificity is important to prevent false positive test results. A *false positive* is a result that incorrectly indicates the presence of the condition being tested for. For example, if someone received a positive COVID test result, when in fact, they were not infected with the SARS-CoV-2 virus.

Ideally, a diagnostic test would have both high sensitivity and high specificity, but as sensitivity increases, specificity typically decreases and vice versa. Selecting the balance between sensitivity and specificity depends on what the test is being used to diagnose.



Types of COVID-19 tests

There are two general methods for testing whether a person is or has been infected with a virus: antibody tests and viral tests. *Antibody tests* detect antibodies in your blood produced in response to infection with the SARS-CoV-2 virus or a vaccine. Antibody tests will tell you if you have been exposed to a virus or vaccine in the past, but they cannot be used to diagnose an active infection. On the other hand, *viral tests* (also called diagnostic tests) detect an active infection with the SARS-CoV-2 virus and are used to diagnose patients. There are two main classes of viral tests: *nucleic acid tests* and *antigen tests*. These are described in the table below.

Viral tests (also called diagnostic tests) identify active viral infections. There are two main types of viral test: nucleic acid tests and antigen tests.

	Nucleic acid tests detect viral genetic material	**Antigen tests** detect viral antigens, molecules on the surface of the virus
How long does it take to get results?	• As quickly as under an hour if testing performed on-site • More typically a few days, as samples are usually sent to a testing lab	• Results typically reported in minutes
Pros	• Additional testing typically not needed to verify test results • Highly sensitive—can detect early infections • High specificity	• Faster • Easier to perform • Less expensive • High specificity
Cons	• More expensive • Require expensive specialized equipment	• Less sensitive—may not detect an early or late infection • Additional nucleic acid test may be required to verify negative test results

Rapid tests (also called point-of-care tests) are diagnostic tests that are performed where the sample is collected from the patient, with results ready in minutes or hours rather than days. Rapid tests exist for both nucleic acid tests and antigen tests, although only antigen tests are available for at home self-testing.

You have probably also heard about variants of the virus that causes COVID-19. *Variants* are versions of the virus that have acquired mutations in the viral genome. Some variants may be more infectious or more deadly than the original form of the virus, so people are often not just concerned with whether a patient is infected, but also want to know which specific variant of the virus is responsible for the infection. Standard testing only detects the presence of viral genetic material and cannot identify which variant of the SARS-CoV-2 virus a person is infected with. To identify variants, viral genetic material is amplified using PCR, and then typically sequenced. Sequencing reads each of the nucleic acid bases or building blocks of the viral genetic material, allowing scientists to compare with other viral sequences and identify variants. Sequencing is usually only performed in certain cases or only on a select subset of tests so that public health officials may monitor which variants are spreading in the community.