NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

The Power of a Test:

How COVID-19 Is Diagnosed and Who Does It

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Part I –The Clinical Laboratory

Marcus stepped out of his car and into the sunshine, ready to head into the hospital for another day of work in the molecular diagnostics laboratory. Having graduated six months ago from college, he felt very fortunate to have found a great job that would give him valuable experience until he was ready to apply to medical school. Marcus had majored in biology and spent his senior year working in a research lab studying the genes that control early embryonic development in zebrafish. In his current job, he was studying genes again, but this time for a completely different purpose: to find genetic changes in patient DNA that could be contributing to their disease. Entering the laboratory wing of the hospital, Marcus thought, *Working in the molecular diagnostics lab sure is different from when I was doing experiments in the research lab!*

What made Marcus think that? What exactly is a molecular diagnostics lab, and how does it differ from a research lab?

A molecular diagnostics laboratory is just one of several types of laboratories that exist in hospitals. These laboratories are also referred to as medical laboratories, or clinical laboratories. The term "clinical" refers to the actual diagnosis and treatment of patients.

The following resources may help you to answer the questions below.

- Food and Drug Administration (FDA). Tests used in clinical care. <https://www.fda.gov/medical-devices/vitro-diagnostics/tests-used-clinical-care>
- American Association for Clinical Chemistry (AACC). Where lab tests are performed. <https://labtestsonline.org/articles/where-lab-tests-are-performed>
- American Association for Clinical Chemistry (AACC). Collecting samples for laboratory testing. <https://labtestsonline.org/articles/collecting-samples-laboratory-testing>

Questions

1. What types of samples might be collected from patients to use for laboratory testing?

2. What are some examples of situations where a doctor might order a lab test to aid in the diagnosis and treatment of a patient?

There are many rules and regulations in place in order for a clinical laboratory to conduct testing. All clinical laboratories are regulated by the government, through the Clinical Laboratory Improvement Amendments, or CLIA. All clinical laboratories must be CLIA-certified. To obtain certification, a laboratory needs to provide evidence that the tests that they perform meet quality control standards, that the personnel performing the tests are adequately trained, and that the equipment being used for the test is functioning correctly and is properly calibrated.

The following resources may help you to answer the questions below.

- American Academy of Family Physicians (AAFP). Clinical Laboratory Improvement Amendments (CLIA). <https://www.aafp.org/practice-management/regulatory/clia.html>
- Food and Drug Administration (FDA). Clinical Laboratory Improvement Amendments (CLIA). <https://www.fda.gov/medical-devices/ivd-regulatory-assistance/clinical-laboratory-improvement-amendments-clia>
- Centers for Medicare and Medicaid Services (CMS). How to obtain a CLIA certificate. <https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/HowObtainCLIACertificate.pdf>

Questions

3. Why does the government regulate clinical laboratories? Why is CLIA certification required?

4. How does the work of a clinical laboratory differ from the experiments being conducted in a research laboratory? Why don't research laboratories have to comply with CLIA regulations?

Part II – Diagnostic Testing

For weeks, Marcus had been checking the Centers for Disease Control and Prevention (CDC) website in the evenings after work. The virus, referred to as SARS-CoV-2, was spreading across the globe and seemed to be taking hold in the United States, with cases of the associated respiratory disease COVID-19 increasing daily. The World Health Organization (WHO) had officially classified the outbreak as a pandemic, and large numbers of cases were being reported in the state where Marcus lived.

When he arrived for work this morning, the laboratory director, Dr. Elaine Cordozo, called an emergency staff meeting. When everyone had gathered in the conference room, she made an announcement. "Working with our state's health department, we have been given the important job of serving as a sample processing and testing laboratory for COVID-19 infection. We will begin preparations for conducting this testing immediately and will commence testing of patient samples as soon as possible."

The next several days were a blur for Marcus and his colleagues. They worked quickly to get the laboratory testing set up. Finally, the day arrived that they would commence with COVID-19 diagnostic testing.

How does COVID-19 testing work? How do healthcare professionals make a diagnosis of COVID-19 infection? Let's follow Marcus through the process.

Read the following graphic article to learn about the SARS-CoV-2 virus and how it infects the human body:

- Corum, J. and C. Zimmer. 2020. How coronavirus hijacks your cells. *The New York Times*. <https://www.nytimes.com/interactive/2020/03/11/science/how-coronavirus-hijacks-your-cells.html>

Watch this video to learn about the COVID-19 testing process:

- The Jackson Laboratory. 2020. COVID-19 testing process. Running time: 1:15 min. <https://youtu.be/ORRLyCZpIus>

Carefully read the CDC test protocol outlined in Table 1 below to learn about how SARS-CoV-2 is detected after the sample is collected from the patient and received by the clinical lab:

Table 1. Centers for Disease Control (CDC) SARS-CoV-2 coronavirus diagnostic test protocol for CLIA laboratory.

		Purpose	*How it works*	*Notes*
Stage 1	Nucleic acid extraction	To isolate viral RNA from a patient sample	All nucleic acids are first extracted from the sample. Next, viral RNA is specifically isolated from other sources of RNA.	Viral RNA is isolated based on size. Viral RNA genomes are much larger than RNA from other sources: the SARS-CoV-2 RNA genome is ~30,000 bases long; human RNA is ~100s bases long.
Stage 2a	Reverse transcription	To convert viral RNA into cDNA	Viral RNA isolated in Stage 1 is read by a reverse transcriptase enzyme, which works to make complementary DNA (cDNA) copies of the RNA template.	The viral RNA from the patient sample must be amplified by PCR (Stage 2b) in order to be detected. PCR requires a DNA template, therefore, the RNA virus first needs to be converted to DNA.

Stage 2b	Quantitative PCR	To amplify, detect and quantify the virus-specific sequence in a sample	PCR amplification using cDNA generated in Stage 2a as template. Three fluorescent primer probe sets are used to detect distinct regions of the viral genome of SARS-CoV-2. A special PCR machine with a fluorometer detects fluorescence generated when primers find their target sequence and target DNA is amplified. Fluorescence is quantified at every cycle of the PCR process in order to determine the amount of virus present.	As PCR is used to amplify the target sequences, more fluorescent probes can bind to the newly generated PCR products, and more fluorescence is emitted and detected.
Stage 3	Data analysis	To generate diagnostic report	Analyze graphs of quantitative PCR results (fluorescence data and PCR cycle number) for each sample to determine if it is SARS-CoV-2 virus positive, negative, or ambiguous.	The fewer cycles it takes to detect, the more abundant the viral genome is in the sample. Cycle number and level of fluorescence all contribute to interpreting results as strong positives, strong negatives, or ambiguous.

Questions

1. After reviewing the protocol above, what terms have you heard of before?

2. After reviewing the protocol above, what terms are unfamiliar to you?

Read the following graphic article to learn about the SARS-CoV-2 genome:

- Corum, J. and C. Zimmer. 2020. Bad news wrapped in protein: inside the coronavirus genome. *The New York Times*. <https://www.nytimes.com/interactive/2020/04/03/science/coronavirus-genome-bad-news-wrapped-in-protein.html>

Let's take a closer look at the equipment used and at each stage of the protocol to better understand how a sample is processed and analyzed for COVID-19.

General Equipment Used for Sample Preparation

The essential molecular biology equipment used in a diagnostic lab is depicted in Figure 1. After individual patient samples arrive at the diagnostic lab, they are processed in **small tubes** stored on **tube racks**. **Micropipettors** are used to draw up liquid solutions used in the various steps of this protocol. Multiple samples can be handled in parallel together on **microplates** to save time. These plates can hold 96 different samples, and are loaded onto a special **PCR machine** used for the amplification and detection of the target sequence.

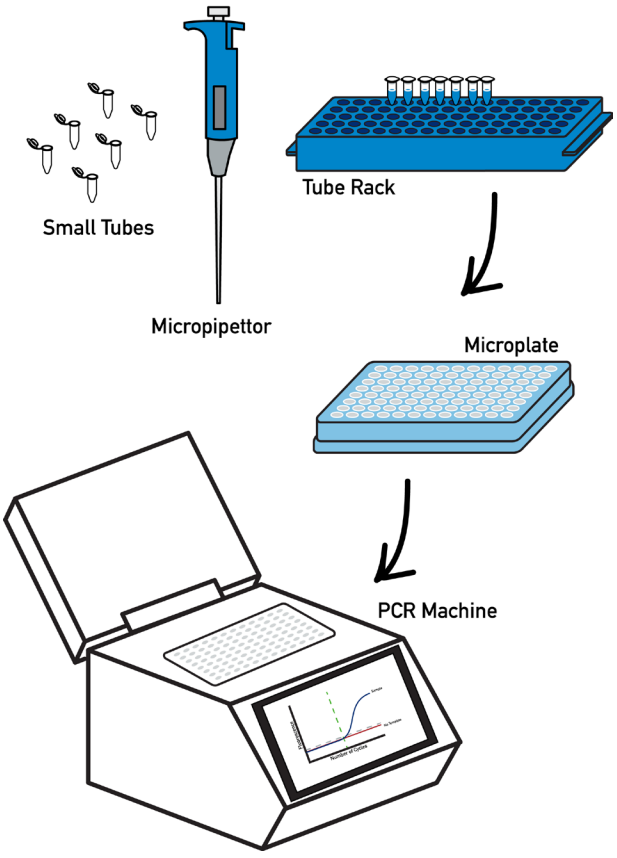


Figure 1. Materials Used in the Diagnostic Lab.

Stage 1 – Nucleic Acid Extraction

After the sample arrives in the diagnostic lab, the first step is to specifically isolate the genetic material of the virus from all of the content found in a patient's sample. In this instance, SARS-CoV-2 uses RNA as its genetic material, so viral RNA must be extracted and isolated. The process is as follows and shown in Figure 2:

1. Obtain the patient sample.
2. Expose all of the genetic material found in the sample.
3. Purify the viral RNA and discard all other contents.

Questions

3. What samples are typically collected from patients suspected of coronavirus exposure?

4. Name three sources of nucleic acids you expect to find in a patient sample.

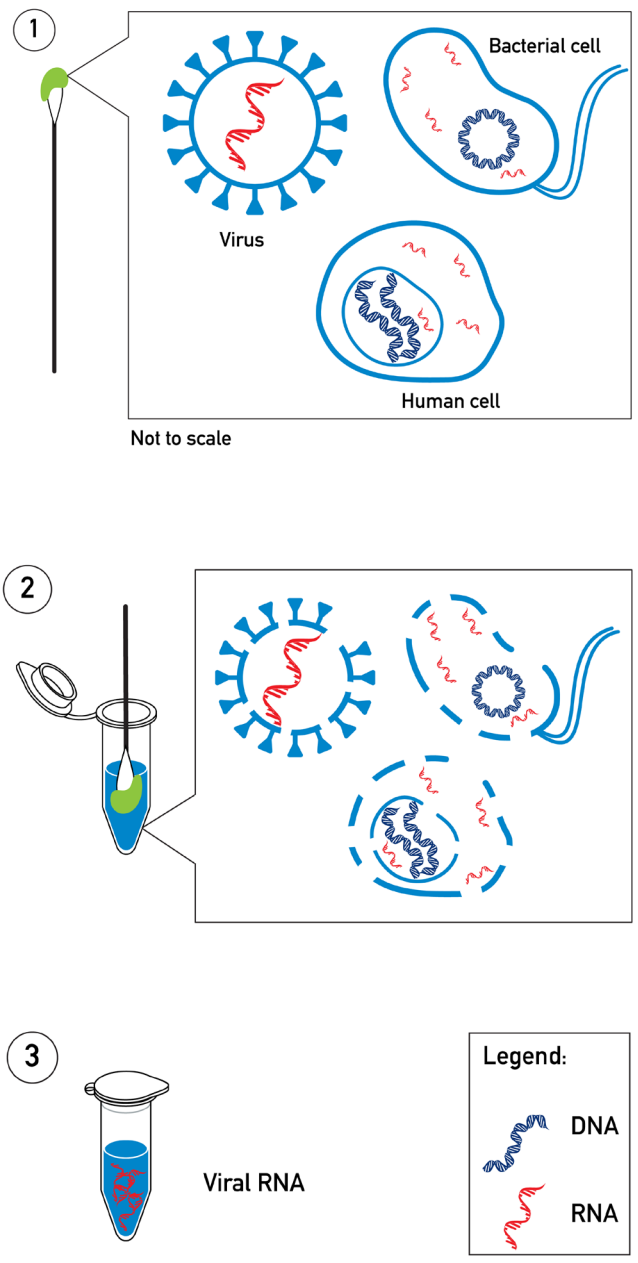


Figure 2. Steps in Processing a Patient Sample. The steps required to isolate viral RNA for diagnostic testing from a patient sample are shown. In step one, the different biological entities obtained from a swab are depicted. In step two, all nucleic acids are released into solution. Step three shows viral RNA, isolated and ready to analyze. Please note the images are not to scale.

Stage 2a – Reverse Transcription (RT)

Once the RNA from the virus is isolated, it is ready to be amplified, detected, and analyzed. The first half of Stage 2 (Stage 2a) requires the viral RNA to be converted to complementary DNA copies, called cDNA for short. This process of making a DNA copy of RNA is called reverse transcription. This is accomplished by a special enzyme called reverse transcriptase, which is not normally found in living cells. Reverse transcriptase was actually discovered in, and isolated from, RNA viruses. The process is as follows and is shown in Figure 3:

1. The viral RNA isolated from Stage 1 is in a solution.
2. The solution contains short single-stranded DNA oligonucleotides, called primers, that bind to the RNA sequence.
3. The solution also contains free DNA nucleotides (A, C, T, and G).
4. The solution also contains the reverse transcriptase (RT) enzyme. The RT enzyme uses the primers to begin adding nucleotides complementary to the RNA.
5. The RT enzyme works along the RNA template, reading the RNA sequence and adding the complementary DNA nucleotides.
6. The RT enzyme makes an exact DNA copy of the RNA template. It does this for all RNA present.
7. The cDNA is now ready for amplification by PCR.

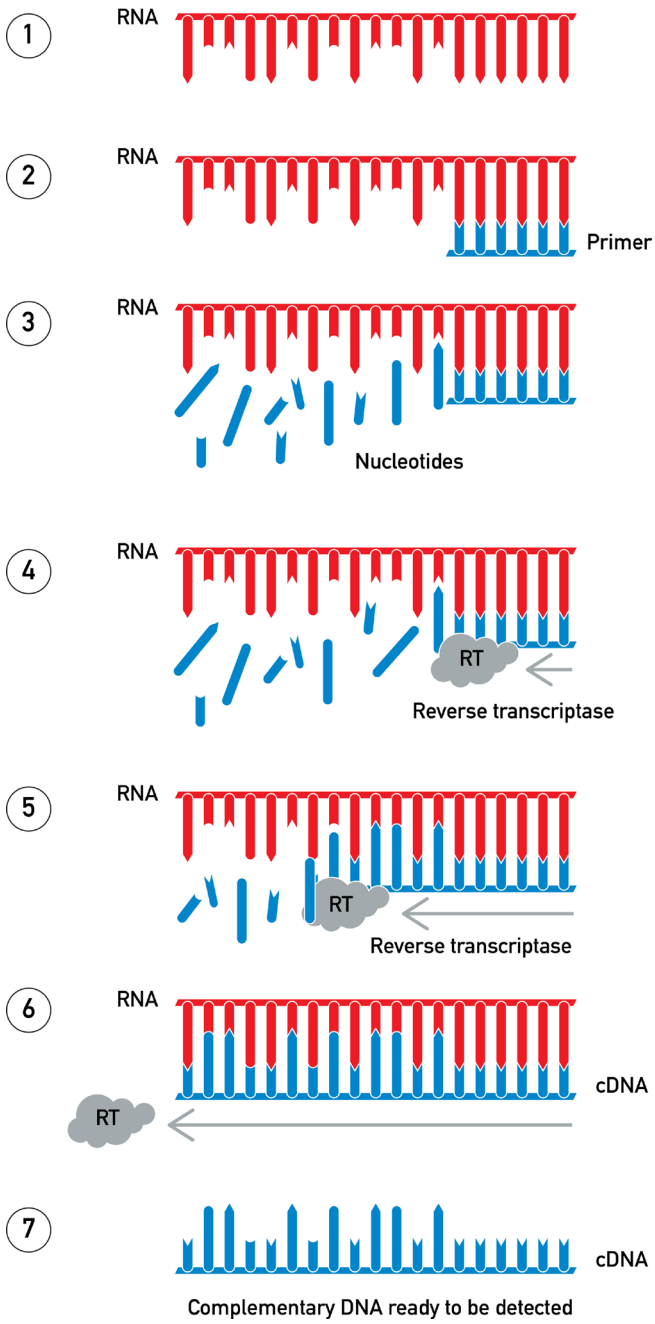
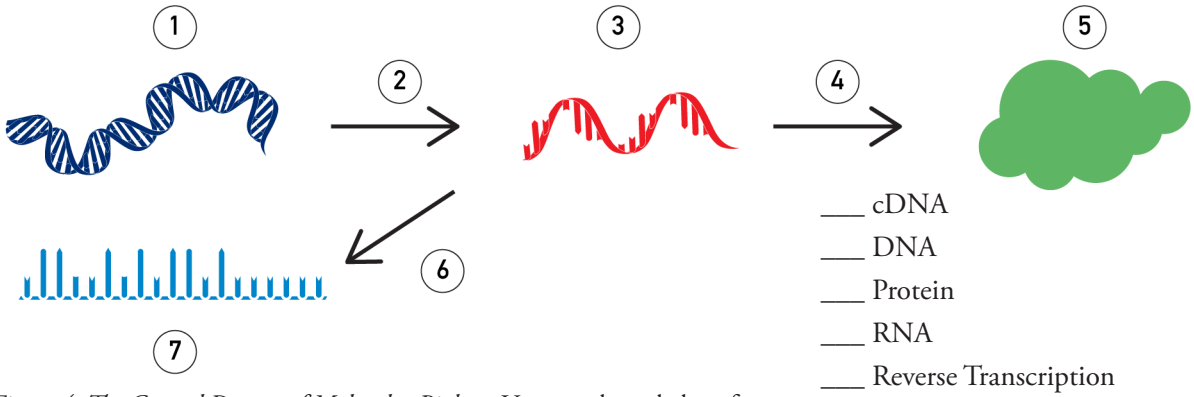


Figure 3. Steps in Reverse Transcription (RT). The molecular biology steps in the process of reverse transcription (RT) leading to the generation of complementary DNA (cDNA) from an RNA sample are shown. This process allows for the amplification of the sample in Stage 2b, quantitative PCR (qPCR).

Activity 1

Recall the central dogma of molecular biology: *When DNA is made into RNA it is called transcription. When RNA is made into protein it is called translation.* Where does reverse transcription fit in this process? Match each keyword to the number in Figure 4 below.



___ cDNA
___ DNA
___ Protein
___ RNA
___ Reverse Transcription
___ Transcription
___ Translation

Figure 4. The Central Dogma of Molecular Biology. Use your knowledge of the central dogma to match each term to the numbers in the figure and identify how reverse transcription (RT) fits into this process.

Stage 2b – Quantitative PCR

Watch the following video about quantitative PCR (qPCR):

- *Overview of qPCR.* Produced by New England Biolabs, 2016. Running time: 2:44 min. <https://youtu.be/1kvy17ugI4w>

The second part of Stage 2 serves to amplify and detect the cDNA generated in Stage 2a. Like traditional PCR, quantitative PCR (qPCR) cycles through temperatures in order to amplify few DNA copies into many DNA copies. Unlike traditional PCR, qPCR is able to determine exactly how many copies of DNA are present in the sample. The process is as follows and is shown in Figure 5:

1. The cDNA generated in Stage 2a is used as a template for the qPCR amplification. Primers find the target sequence to help the polymerase start to amplify and make more copies. Fluorescent probes are in the solution that will detect the target sequence as more copies are made during each cycle of the PCR.
2. As in a traditional PCR, the target sequence is amplified exponentially.
3. As more copies of the target sequence are made, there is more chance for the fluorescent probes to bind. This fluorescence is detected by the fluorometer inside the qPCR machine. Since the cDNA is a DNA copy of the RNA from the virus, the more viral sequence in your sample, the more fluorescent probes will bind. With every cycle you will detect more fluorescence.

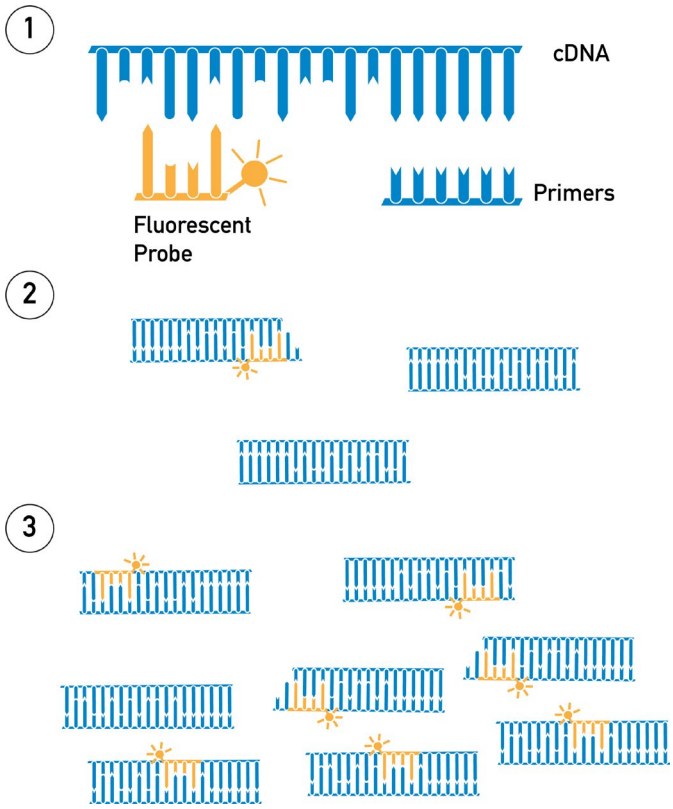


Figure 5. Steps in Quantitative PCR (qPCR). The steps in the process of the quantitative PCR (qPCR) assay are shown. This process leads to the amplification and quantification of a cDNA sample.

Questions

5. Compare and contrast traditional PCR with quantitative PCR.

6. How can we ensure we are only amplifying and detecting viral sequences, and no other sequences that may be in the tube?

Stage 3 – Data Interpretation

The graph below in Figure 6 shows the type of data collected from a quantitative PCR (qPCR) assay. Let's break down the components of the graph to analyze the results.

- Fluorescence (*y* axis) is tracked and reported for each sample across all cycles of the PCR assay (*x* axis).
- The threshold of detection (Line D) is the amount of fluorescence needed to distinguish true fluorescence signal from background signal from the assay.
- A positive control (Sample A) is included as a reference for what a virus-containing sample should look like; positive samples should create a high fluorescence signal, far above the threshold of detection.
- A negative control (Sample E) is included as a reference for what a sample without virus should look like; negative samples should yield a low fluorescence signal, below the threshold of detection.
- In this graph, three patient samples (Sample B, Sample C, and Sample F) illustrate different outcomes from this assay.

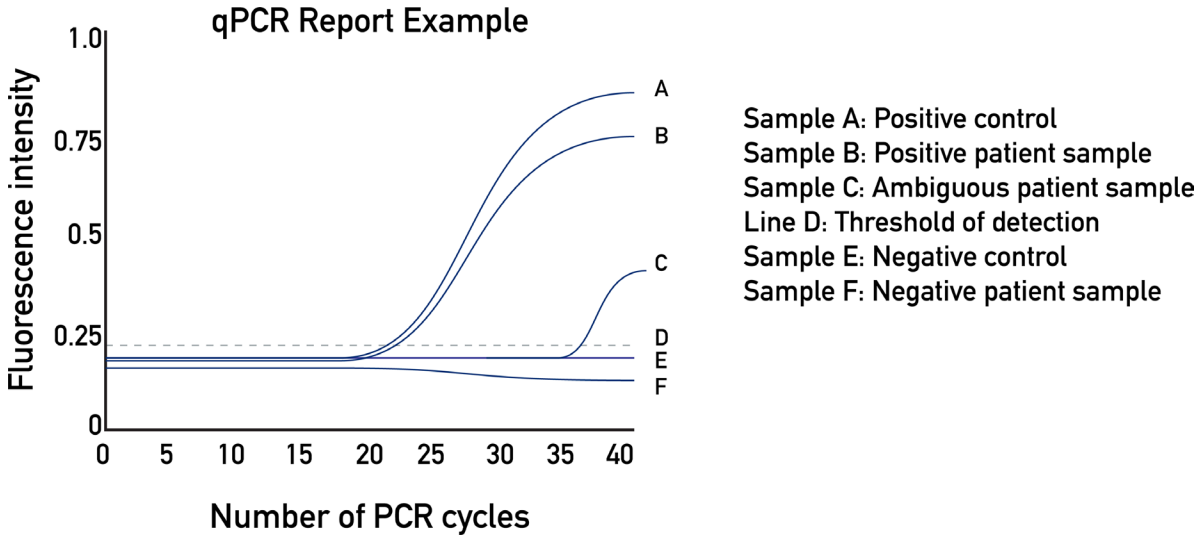


Figure 6. Quantitative PCR (qPCR) Report. Depicted here is an example of a qPCR assay report. This is the data that needs to be interpreted for diagnosis. A positive control sample is used to determine the fluorescence intensity of a known sample. A negative control sample is used to determine the fluorescence intensity of a sample that is known to have no viral RNA. The fluorescence of a patient sample can be compared to that of the control samples to determine if the sample is positive or negative. Sometimes, sample fluorescence can be intermediate, which is interpreted as an ambiguous patient sample (unsure if the sample is positive or negative).

To summarize, let's look at two patient samples and follow the laboratory process, as depicted in Figure 7:

1. Two samples are received in the lab. Viral RNA is isolated from each.
2. The RNA is converted to cDNA via reverse transcription. The cDNA is amplified via quantitative PCR and the fluorescence from the primers is detected.
3. Data is graphed from the qPCR assay. Sample 1 shows a high signal detected early in the cycle run, similar to the positive control. Sample 1 is reported as SARS-CoV-2 positive. Sample 2 shows a low signal below the threshold of detection, similar to the negative control. Sample 2 is reported as SARS-CoV-2 negative.

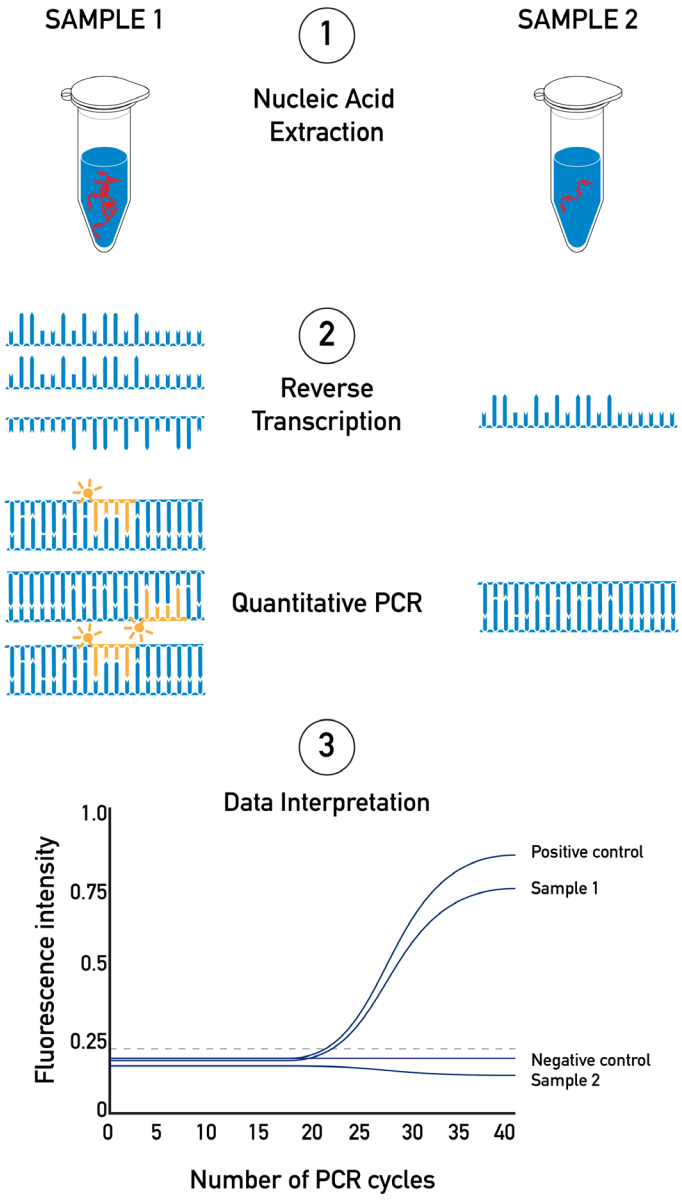


Figure 7. Laboratory Testing Process for Two Different Patient Samples. Depicted here is an example of diagnostic sample processing for two different patient samples. In Stage 1, notice the amount of RNA in each tube is different. In Stage 2, notice the amount of cDNA that is synthesized during the RT step is different. In Stage 3, notice the amount of amplification during the qPCR varies. Due to these factors and other variables in sample processing, you can see that the diagnostic result for each sample is quite different.

Activity 2

Figure 8 shows three examples of quantitative PCR results from three different patients. Use what you have learned about the negative control, positive control, and threshold of detection to label the lines. Notice the difference in the number of cycles (*x* axis) that each sample takes to peak in its fluorescence (*y* axis). Using your knowledge, interpret results of each test by indicating "positive," "negative," or "ambiguous" on the lines provided below.

Patient #1 Result: _____

Patient #2 Result: _____

Patient #3 Result: _____

Question

7. What could contribute to ambiguous results from samples? *Hint:* Think about differences in the viral load (the total amount of virus inside a person) between people, differences in sample collection methods, the differences in sample preparation methods. Can you suggest two factors that may contribute to an ambiguous test result?

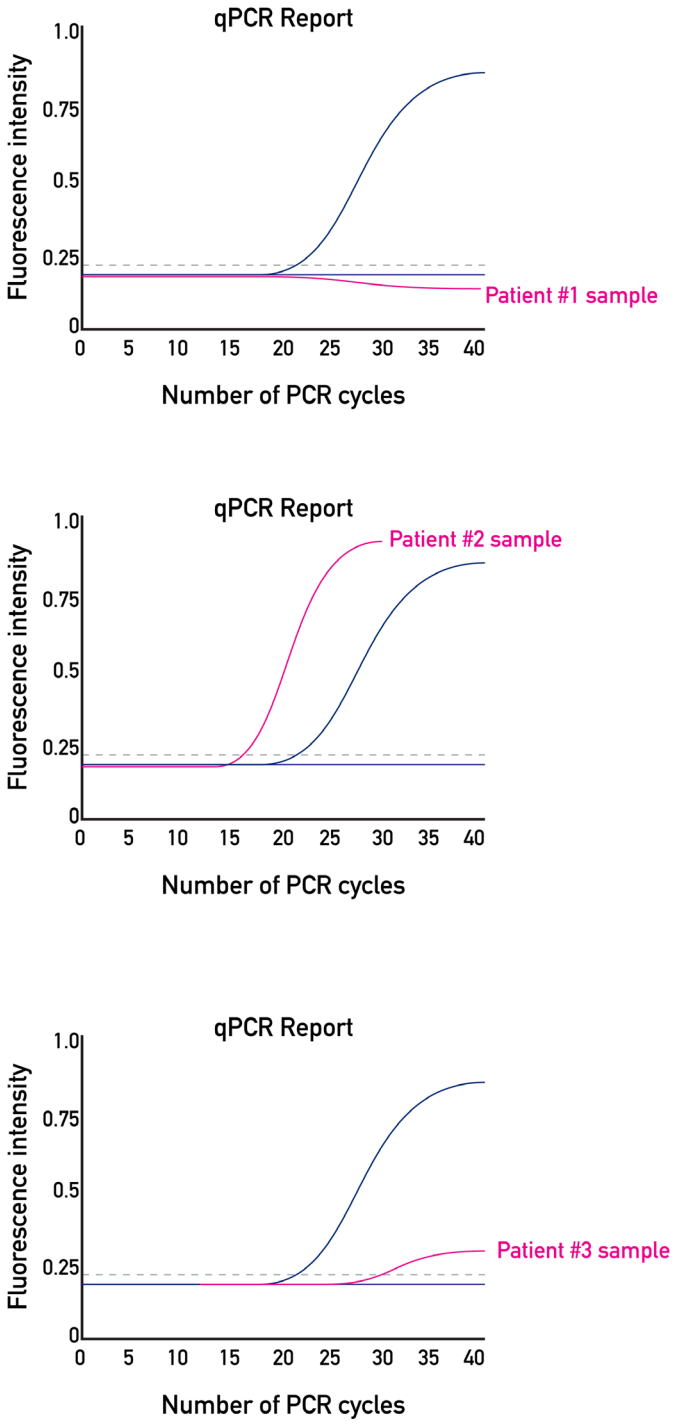


Figure 8. qPCR Reports for Three Different Patient Samples. Use your knowledge of the diagnostic testing process to make a diagnosis in the three patients.

Part III – Careers in a Clinical Laboratory

Marcus was relaxing. He sat on the beach, watching people walking their dogs on a warm spring day. It had been a little over a year since the COVID-19 pandemic that had totally upended his life, both at work and at home. Those were long, busy days at the laboratory, but Marcus was really proud of the work he had done. He always thought that he would find purpose and fulfillment in helping others by becoming a doctor. What he discovered was that he did not need to wait for that fulfillment; he had directly contributed to saving thousands of lives during a major public health crisis. Marcus thought about all of the tests that his laboratory had run to diagnose COVID-19 infection in tens of thousands of patients. The results of the tests that he performed were reported to physicians who then used that information to more effectively treat their patients. Those that tested positive for COVID-19 were instructed to self-quarantine if their symptoms were manageable and to monitor their symptoms closely, seeking medical care if their symptoms worsened. The knowledge of COVID-19 test results led to reduced spread of the virus and quicker hospital care for those who needed it. That realization caused him to smile as he took in the fresh ocean breeze.

We know that doctors save lives, but what about all of the hospital laboratory workers, people like Marcus, who do this every day behind the scenes? It is estimated that 70% of medical decisions depend upon laboratory test results (CDC, 2018, <https://www.cdc.gov/csels/dls/strengthening-clinical-labs.html>). Let's explore career paths in the clinical diagnostic laboratory.

Use the following resources or other fact-based sources of your choosing to answer the questions below.

- Coordinating Council on the Clinical Laboratory Workforce. Careers.
  <http://www.laboratorysciencecareers.com/careers-in-laboratory-science.html>
- Zippia. Resarch career options.
  <https://www.zippia.com/careers/>
- Indeed. Search and compare salaries.
  <https://www.indeed.com/salaries>
- American Academy of Family Physicians. (AAFP). Personnel requirements.
  <https://www.aafp.org/practice-management/regulatory/clia/personnel.html>

Questions

1. Make a list of diagnostic laboratory careers. Who would be critical workers in the journey of a sample through the laboratory? Don't forget those who keep the laboratory running by doing important support jobs. You should name at least five different careers and describe each position.

2. Select two of the careers you listed in Question 1. For each career, provide:
   a. The minimum degree requirements, certifications and/or trainings required.
   b. The salary ranges.

3. What additional degree(s), certifications and/or training would Marcus need to grow in his career?

4. Imagine yourself in one of the careers listed above. Describe your qualifications and what your day to day job might look like.