NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Put to the Test:

RT-PCR and Antibody Detection Diagnostic Tools for COVID-19

by

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Part I – The Beginning of the Outbreak

March 4, 2020

"Wow, busy day in the ER! What is it, a full moon?" Abe, an emergency room physician at Mass General Hospital, looked around in disbelief.

"It's definitely *something!* I can't believe how nuts it is in here." Manda, the attending resident, had been seeing patients all day. "It's weird… they all seem to have the same thing."

In the last four hours, twenty-one people had been admitted to the hospital in similar states. Abe started reviewing the charts, looking for a connection. Each person complained of chest pain, trouble breathing and a painful cough. Several had fevers and some complained of fatigue. Abe considered those details thoughtfully, but also knew that some of those symptoms aligned reasonably well with the flu. But twenty-one at once? And all at this level of severity? This wasn't like most flu outbreaks he had seen. These symptoms sounded a lot like those described from the novel coronavirus outbreak that had occurred in December of 2019 in China. The disease this virus caused, COVID-19, had become a major problem not only in China, but in Europe too. So far, he thought, the only cases in the United States were from people traveling from those regions.

Abe went through their intake interviews, focusing on recent travel history. Fully expecting to find that these patients were coming from China and Europe, Abe was surprised to find that they were not. He did notice something interesting, though. Many of the patients had indicated that they worked for a company called Biogen. Although the nature of their jobs at Biogen were different, they had all attended a local executive conference several days prior. Abe had a sinking feeling as he considered the possibility that COVID-19 was spreading in Massachusetts. But was this really COVID-19?

Abe quickly pulled up his laptop and began looking for information about COVID-19 and the coronavirus that caused the disease. He found that very early reports of the virus called it 2019-nCoV while publications from the last few days used the nomenclature SARS-CoV-2. He decided if he was going to figure out if his patients really did have COVID-19, he should start from the beginning and read the article about patient zero in the United States.

- Holshue, M. *et al.* 2020. First case of the novel coronavirus in the United States. *The New England Journal of Medicine* 382: 929–36. <https://doi.org/10.1056/NEJMoa2001191>.

Questions

Using the article provided, answer the following questions.

1. Where did COVID-19 first emerge?

2. What is a zoonotic virus? Why is SARS-CoV-2 thought to be a zoonotic virus?
3. When and where was the first COVID-19 patient observed in the United States?
4. What were the symptoms of this first patient in the United States?
5. What diagnostic tests were performed on this patient to determine if he had COVID-19? Which test determined a positive COVID-19 diagnosis?
6. What is the mechanism of transmission of COVID-19?
7. Do you think Abe's patients contracted COVID-19 from patient zero in the United States? Why or why not? Speculate as to how Abe's patients may have gotten the virus.

Part II – Exploring the Genome of SARS-CoV-2

As Abe began to learn more about COVID-19, he became increasingly convinced that his patients had it. But who at the conference had given it to them? How did they infect so many others, and did they even know they were doing so? In order to understand the transmission and mechanism of infection of the disease, he needed to understand the SARS-CoV-2 virus that was responsible.

He found Figure 1 illustrating the genome of the virus.

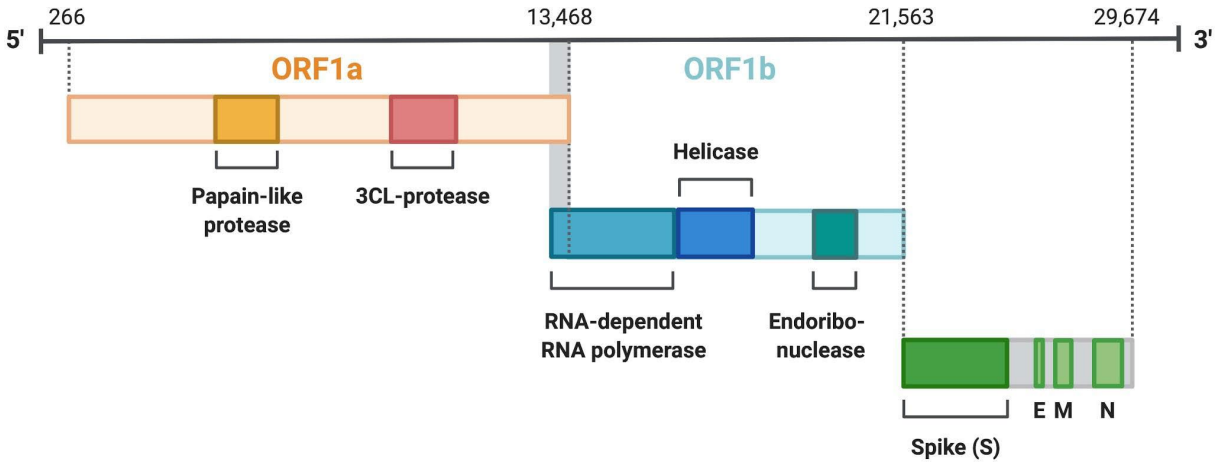

Figure 1. Genome of SARS-CoV-2. Figure made in Biorender.

He was intrigued. What was encoded in this genome? Why was this virus so successful at infecting so many people? What is the evolutionary history of this virus? Clues to these questions can often be ascertained by a close examination of the viral genome and comparing it to other viral genomes. He found several articles analyzing SARS-CoV-2 and related strains, one of which is below:

- Wang, C. *et al*. 2020. The establishment of reference sequence for SARS-CoV-2 and variation analysis. *Journal of Medical Virology* 92(6): 667–74. <https://doi.org/10.1002/jmv.25762>.

Questions

Using the article provided above, answer the following questions.

1. What proteins are encoded in the SARS-CoV-2 genome? What is the general function of each?
2. What are genomic hotspots? Considering COVID-19 is caused by a novel virus among humans, what process(es) might these areas of the genome be critical in enabling?
3. Through what mechanisms do viruses evolve? Does a change to the nucleotide sequence necessarily confer a change to host integration, infection rate or transmissibility? Why or why not?
4. Compare the RNA-dependent RNA-polymerase utilized by SARS-CoV-2 and DNA-dependent DNA-polymerase as in classic central dogma. Which has higher fidelity and why? You may use Pubmed to find additional sources if necessary.
5. Accumulation of favorable mutations is the basis for evolution. Relate the error-prone nature of SARS-CoV-2 RNA replication machinery to the transmissibility of COVID-19 among Abe's patients.
6. What genomic evidence is there to suggest SARS-CoV-2 is a naturally occurring virus that arose via natural selection and was then transmitted to humans?

Part III – Using RT-PCR to Test for COVID-19 Infection

By this point Abe was nearly certain that his newly admitted patients had COVID-19. If this was true, it would mean that the United States would need to figure out who had it and stop the transmission as soon as possible or else…. He shuddered. He knew he needed to test these patients immediately. He began looking into the test available for diagnosis of the disease available from the Centers for Disease Control and Prevention (CDC). He found the official guidelines for *in vitro* diagnostics of this virus in the following document:

- CDC 2019-novel coronavirus (2019-nCoV) real-time RT-PCR diagnostic panel for emergency use only. Instructions for use." <https://www.fda.gov/media/134922/download>.

He remembered that the first COVID-19 patient in the United States (patient zero) had been diagnosed using RT-PCR. This kit was just what he needed! If he could collect samples from his patients and then use RT-PCR, he could definitely diagnose whether or not they had the disease.

Abe remembered learning how to collect samples to test for upper respiratory infections and winced. Patients were usually caught off-guard at how uncomfortable it was and scowled at him afterwards. Regardless, he knew how important specimen collection was to get a reliable test result and found the following resource from Children's Hospital in Colorado to help Manda and the staff collect appropriate samples:

- Nasopharyngeal swab. <https://www.childrenscolorado.org/globalassets/healthcare-professionals/nasopharyngeal-swab.pdf>.

Abe sent his staff off to collect samples and started scrolling through the pages of the CDC PDF. It was dense. "Oh boy," he thought to himself, "RT-PCR, huh?… It's been a while." He needed to brush up on this technique.

Questions

Using the resources provided above, answer the following questions.

1. When using RT-PCR, which biological macromolecule is used to confirm COVID-19 in a patient? What is the source of this biological macromolecule?
2. What is the preferred method of collecting the initial patient sample for diagnostic COVID-19 testing? What considerations need to be taken into account when deciding on which specimen type to collect?
3. In RT-PCR, what does the enzyme reverse transcriptase do? Why is this step necessary for subsequent detection of the virus using PCR?
4. What is RNAse P and why is it used as a control in this assay? What is the source of this biological macromolecule?
5. What are the positive and negative controls used in this diagnostic test and why are they required for interpretation?
6. A primer and probe set is provided for each of the targets tested in this kit. Explain how the primers and probes contribute to amplification and detection of their specific targets.
7. How does the amount of fluorescence in the reaction correlate to the amount of target sequence? Why is there no fluorescence in the absence of target sequence?
8. What does LoD stand for and what does it mean?
9. In the Limitations section of the CDC document the authors state "If the virus mutates in the rRT-PCR target region, 2019-nCoV may not be detected or may be detected less predictably." Why might this be the case? What alterations would you need to make to the components of this test to detect a mutated form of this virus?
10. Why do you think the CDC is using an RT-PCR based test as the primary clinical diagnostic method of establishing COVID-19 infection? Why was it possible for the RT-PCR based test to be developed so quickly at the start of the pandemic?
11. Suggest an explanation for why using this kit may not be feasible for on-site use in all hospitals or clinics. What would limit the number of tests that could be performed in a given time period?

Part IV –Is There Any Other Way? Using Antibodies to Test for Coronavirus

A few days later, Abe burst through the door of his shared office with Manda. "Hey!" he cried, "I was right!" He had just gotten the RT-PCR test results from the first group of patients. "These patients all have COVID-19!" Manda looked up, exhausted. The last few days had been grueling, and it seemed like the waiting room never emptied. Usually she would come up with a snarky retort to Abe's exclamation of being right, but not today. It was clear that all these patients were suffering from COVID-19 and the hospital had nowhere near enough tests to confirm diagnosis for all of them. "Well, I'm glad we can tell those patients what they have, but what about the others?" She motioned toward the waiting room. "We're almost out of tests. Do you have any idea if we're getting more? What are we supposed to do?"

Abe knew that serological tests had been used to study other coronaviruses and wondered if this was possible with SARS-CoV-2.

In a serological test a sample of a patient's blood is collected and the serum, or clear liquid part, is separated from the clotting factors and cells. The blood serum contains liquid plasma, proteins not-involved in clotting, electrolytes, hormones, other water-soluble substances, as well as foreign antigens. One class of proteins found in the serum are antibodies. Antibodies are proteins made by a person's immune system that bind to, neutralize, and/or tag a foreign pathogen for degradation. In a diagnostic serological test, clinicians look for the presence of antibodies to a foreign pathogen to confirm that a patient has been infected with the virus.

The presence of antibodies that recognize antigens of SARS-CoV-2 would indicate a person had been infected with the virus and their immune system had mounted a response. Thinking of the structure of the coronavirus (Figure 2), Abe considered various antibody targets and how they might serve as possible targets for a serological test.

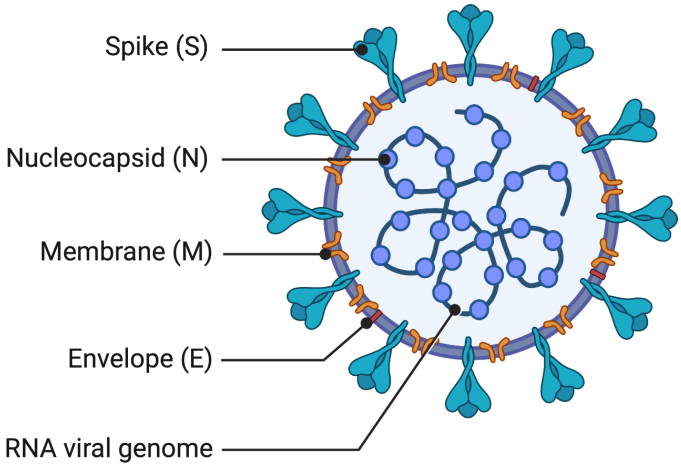

Figure 2. Structure of SARS-CoV-2. Figure made in Biorender.

Questions

1. What is an antibody? What does an antibody look like? What components does it have? Where and how does it bind to the antigen?
2. What is an antigen? What does an antigen look like? Where does it come from?
3. Describe the process of human antibody production. In an ideal situation, why doesn't an antibody identify self-targets?
4. How long does a person express antibodies for a target antigen after infection? Will a patient continue to make antibodies for a virus even after the infection has cleared? Why or why not?
5. Considering the structure and composition of SARS-CoV-2, list possible antigens for antibody binding. Which one do you think would be the most effective for a diagnostic serological assay? Be prepared to defend your choice to the class.

Part V – Finding an Antibody that Binds SARS-CoV-2

Abe began looking up different antibodies for serological testing for SARS-CoV-2 and, much to his excitement, he found two antibodies that were already available for use for detection of the spike protein of SARS-CoV, which caused the 2003 outbreak. Both SARS-CoV and SARS-CoV-2 use their spike protein to bind to the ACE2 receptor on target cells to allow for entry and infection. One antibody he identified bound to the ACE2 binding site on the SARS-CoV spike protein and the other antibody bound elsewhere on the spike protein.

He ordered a batch of both antibodies from a nearby company, and Abe began testing each to see if either could bind to SARS-CoV-2. "If this works, we could get all these patients tested in no time. Ok… what am I going to test these antibodies out on?" Luckily, a neighboring lab had been able to produce the virus in tissue culture cells and gave him a sample. Much to his dismay, although the first antibody detected SARS-CoV, it showed no affinity for binding SARS-CoV-2. "Ugh," Abe muttered. "Maybe this wasn't such a great idea." He checked the second antibody. It showed high affinity for SARS-CoV-2. "Yes! Maybe this will work, after all!" Abe always loved being in the lab. After the last few weeks in the ER, he was toying with the idea of a career change.

Questions

Use the paper below and Pubmed to answer the following questions.

- Tian, X., *et al.* 2020. Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. *Emerging Microbes and Infections* 9(1): 382–5. <https://doi.org/10.1080/22221751.2020.1729069>.

1. How similar are the spike proteins from SARS-CoV and SARS-CoV-2?
2. What does ACE2 normally do? Where is it normally found in the body?
3. How does ACE2 facilitate entry of SARS into the cell?
4. Provide a plausible explanation for why Abe's first antibody bound SARS-CoV spike protein but not SARS-CoV-2 spike protein.
5. What does it mean that the second antibody bound SARS-CoV spike protein with high affinity? Why is this important with respect to how the antibody functions in the body?

Part VI – Designing a Serological Assay for SARS-CoV-2

Feeling invigorated after finding an antibody that bound SARS-CoV2, Abe got to work designing a new test. Abe knew that many serological tests utilize a technique called an enzyme-linked immunosorbent assay (ELISA). Specifically, many use a sandwich ELISA which is shown in Figure 3.

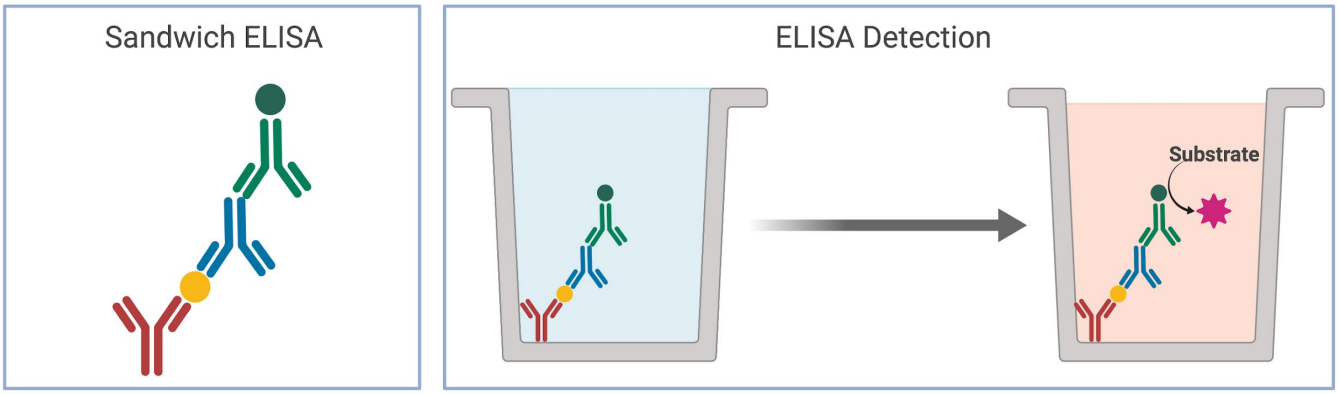

Figure 3. Sandwich ELISA. Figure made in Biorender.

A sandwich ELISA requires three antibodies and an antigen. The red antibody is called the capture antibody, which is known to bind to the antigen of interest. Abe knew this antibody had to bind SARS-CoV-2 with high affinity, which was why he was so excited to find an antibody that did so. The yellow sphere is the antigen of interest, and in this case, it would be some part of SARS-CoV-2 spike protein. The blue antibody is the primary antibody that also binds the antigen of interest, and in this case, this antibody would come from the serum of a suspected SARS-CoV-2 infected patient. The green antibody with the attached green sphere is the detection antibody that can bind to all human antibodies. In other words, the detection antibody targets another antibody as its antigen. The detection antibody is an engineered antibody because the green sphere is an enzyme that is covalently attached to the antibody. The pieces of spike protein and the detection antibody can be purchased commercially.

The assay Abe wanted to use works as follows. First, a test tube or microplate is coated with capture antibody. Recombinant purified spike protein is then applied. This allows the antigen (spike protein) to bind to the capture antibody. The test tube is washed extensively to remove any unbound spike protein. Next, the serum of a patient suspected to be infected with SARS-CoV-2 is applied. If the patient has antibodies in their serum that bind to spike protein in the body, these antibodies will also bind to the spike coating the test tube. These antibodies from the patient are the primary antibodies. This generates the sandwich of capture antibody, antigen, and primary antibody. After the serum is applied, the test tube is washed extensively to remove any unbound biological molecules. Finally, the detection antibody is added and binds the primary antibodies from the serum that are bound to the antigen. Again, the test tube is washed extensively to remove any unbound detection antibodies.

In order to detect if the patient has been infected with SARS-CoV-2, the substrate for the enzyme on the detection antibody is added to the test tube. If the patient is infected with the virus, the enzyme will catalyze a chemical reaction that can be monitored. In the figure above, the substrate is added and, if the enzyme catalyzes the reaction, it causes the solution to turn pink.

Abe was excited about the prospect of using an ELISA test for his patients. Although his preliminary work showed that it was promising, he realized it would take time before an antibody-based assay was fully developed and approved for SARS-CoV-2 infection. For now, he was stuck using RT-PCR to diagnose, but he knew to fully understand how many people have been infected, a serological assay is absolutely required.

Questions

1. If serum from a patient that had not been infected with SARS-CoV-2 was used in this sandwich ELISA, would the solution turn color? Explain your answer.

2. How could a sandwich ELISA differentiate between a patient with many antibodies versus few antibodies? What would this suggest regarding how long the patient had been infected? How would you interpret a very weak signal?

3. Will this assay be useful for someone who has recently been infected? Why or why not?

4. The article below discusses another type of ELISA that has shown promise for detecting patients that have been infected with SARS-CoV-2.

   • Amanat, F., *et al.* 2020. A serological assay to detect SARS-CoV-2 seroconversion in humans. *medRxiv*. Preprint. <https://doi.org/10.1101/2020.03.17.20037713>.

   Abe's sandwich ELISA is more specific than this ELISA. Hypothesize as to why.

5. How could you modify this assay to test for active virus infection, rather than antibodies that demonstrate previous infection? Provide the rationale for each modification.

6. Why is a serological test necessary to determine the total number of people infected with SARS-CoV-2? Why would the RT-PCR assay not work for people who had fully recovered from infection?

Part VII – Where Do We Go from Here?

Over the following two weeks, the number of patients coming into the hospital increased every day. Abe and Manda continued to see friends, family members and coworkers of the original Biogen employees being admitted for care. Presumptive COVID-19 cases unrelated to the Biogen conference were being seen as well. Although all patients were asked to quarantine, the state and the country continued to see the disease spread. It was evident that community transmission was occuring, leading to an exponential increase in cases, and COVID-19 was declared a world-wide pandemic.

Abe began to realize that the only real way to stop this was to create a vaccine and hope for herd immunity. Abe knew that several vaccines for SARS-CoV-2 were being developed by both private and public organizations, but he worried that the timetable to get them to mass production and availability might be too long to help his community. He knew that for now he needed to do all that he could to treat everyone to the best of his ability.

Assignment

You are a scientist at the NIH responsible for allocating grant money for research on a vaccine for COVID-19 during the initial weeks of the United States outbreak. Many groups have requested funding for their individual efforts, and your job is to decide whose research is the most promising and likely to yield quick, effective results.

Proposal 1: A research group in Maryland has proposed using surface-exposed pieces of the S (Spike) protein as potential vaccine antigenic components.

Proposal 2: A research group in California has proposed using polypeptides taken from the N (Nucleocapsid) protein that are essential in binding SARS-CoV-2 RNA as potential vaccine antigenic components.

Proposal 3: A research group in Texas has proposed using lipid constructs taken from the membrane components of SARS-CoV-2 as potential vaccine antigenic components.

Proposal 4: A research group in Massachusetts has proposed using single-strands of RNA coding for the S protein as potential vaccine antigenic components.

Proposal 5: A research group in New York has proposed using sequences of DNA coding for the S protein as potential vaccine antigenic components.

Based on your scientific knowledge on COVID-19 and vaccine types, efficacy and function, select one of the above proposals to fund. Write a letter to the head of the NIH explaining which proposal you are funding and why. Be sure to defend your choice with scientific sources. Consider the following when crafting your letter:

- What is a vaccine and how does it work with respect to disease prevention? How do we ensure a vaccine is specific to a pathogen and does not cause an unwanted immune response?
- What aspect of SARS-CoV-2 does this vaccine target? Where is it found on the virus? Does infection need to occur for this target to be useful? Why is this a reasonable target? What makes this target a better choice than the others suggested?
- What other pathogens are being targeted with this type of vaccine? How are they like or not like SARS-CoV-2?
- How quickly would you expect this vaccine to be available? Why? What is the precedent or basis for this prediction?

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