Written remarks to accompany the testimony of Steven Quay, MD, PhD

“Revisiting Gain of Function Research: What the Pandemic Taught Us and Where Do We Go From Here”
Senate Committee on Homeland Security and Governmental Affairs’
Subcommittee on Emerging Threats and Spending Oversight

Subcommittee Chair Senator Margaret Wood Hassan, Ranking Member Senator Rand Paul, Members of the Governmental Affairs’ Subcommittee on Emerging Threats and Spending Oversight, invited Senator committee participants, Ladies and Gentlemen.

I am honored to participate with my esteemed colleagues, Drs. Ebright and Esvelt, in this forum entitled: “Revisiting Gain of Function Research: What the Pandemic Taught Us and Where Do We Go From Here.”

My prepared remarks will take about seven minutes. I will cover six topics:

- I will begin with an overview of the evidence related to the origin of the pandemic. My conclusion from two years of investigation is that the pandemic began with a laboratory-acquired infection.
- The virus has three genomic regions that have the signature of synthetic biology, that is, gain-of-function research.
- Two of those regions involve the three types of academic gain-of-function research that are permitted
- One region has features of the two types of forbidden gain-of-function research that are associated with bioweapons development, asymptomatic transmission and immune system evasion.
- Finally, I will present evidence of synthetic biology research at the Wuhan Institute of Virology being conducted in low level, BSL 2/3 facilities, in December 2019 on the Nipah virus, which is >60% lethal but is not naturally airborne. This is the most dangerous research I have ever encountered.
- I will close with five recommendations for future gain-of-function research.

Where did the pandemic begin?

The competing hypotheses are a natural spillover at the Huanan Seafood market in Wuhan China and a laboratory-acquired infection, most likely at the Wuhan Institute of Virology or WIV. Before December 2019, the WIV had published over 65% of all coronavirus scientific papers in the world. Until it was removed from the WIV website at 2 am local time, September 19, 2019, they maintained a database of over 21,000 viruses collected over two decades, in part with NIH funding. To my knowledge, no western scientist or organization has had access to this database since the pandemic began.

Two papers published last week by western scientists and a flurry of coordinated news coverage purported to end the debate, stating the pandemic began in the market in December 2019 and even claiming the market contained infected animals. There are at least six problems with these papers:

- No animal has ever been found to be infected with CoV-2. Hundreds from the market were tested and over 80,000 throughout China were all negative.
All environmental specimens from the market were the result of human infection, not animal infection. For comparison, with the 2003 SARS infection >90% of market animals were infected.

These papers suppress cases from the eastern side on the Yangtze River, near the WIV, for no apparent reason. These include cases identified during the World Health Organization investigation.

Scientists agree that the most ancestral version of CoV-2 that infected humans, named Lineage A, did not infect any patient from the market.

Orthogonal methods agree that the virus was circulating in Wuhan in the fall of 2019 and well before the market cases. I, as well as many others, believe the market cases were a super-spreader event.

My own research has identified the earliest cluster of hospitalized patients with both the Lineage A and Lineage B virus at the People’s Liberation Army Hospital in Wuhan, identified on the chart shown here. This hospital is about 3 km from the WIV and along Line 2 of the Wuhan subway system. My research also showed that all early cases were along this same subway line, one of nine in Wuhan, and that the probability this was by chance is one in 68,000. The Line 2 COVID Conduit, as I called it, includes the PLA Hospital, the WIV, the market, and at the last stop, the international airport. You can literally walk down into the subway system and next exit in the world in London, Paris, Dubai, and New York City, all before having any symptoms. In the fall of 2019 one million people a day used Line 2 and modelling by others suggested the pandemic could not have occurred without the spreading impact of Line 2.

What are the gain-of-function features of SARS-CoV-2?

First, gain-of-function research is defined as making artificial changes to a microbe in a laboratory, seeing what new properties it acquires by those changes, and then often performing additional research to find vaccines or therapeutic that can stop this synthetic virus.

So create something that doesn’t exist in nature and see if you can kill it.

The three kinds of gain-of-function research that have been agreed are acceptable in academic work are changing trophism, that is changing the host animal; changing infectivity (ease of transmission) and/or pathogenicity (how dangerous it is). Two kinds of gain-of-function research have been agreed by scientists as off limits, as they have bioweapons features. These are making infections hard to detect and making viruses that can evade the immune system.

SARS-CoV-2 has features of all five kinds of gain-of-function research, including the off-limits work.

Host selection, infectivity, and pathogenicity in SARS-CoV-2 are governed by the two-step verification system used by coronaviruses to infect cells. Step one is the handshake between the receptor binding domain and the human ACE2 receptor on the surface of the human respiratory system. Step two is the spike protein cleavage site, in CoV-2 the so-called furin cleavage site, which puts a cut in the spike protein. At that point the virus injects its genetic material into the cell and begins the 12 hour or so process of replicating. This ends with the cell dissolving and thousands of new viruses being released.
In SARS-CoV-2, the receptor binding domain was largely perfected for human infection. Specifically, the first virus to infect humans had only 17 mutations it could make out of 4000 that would improve the ACE2 handshake. With SARS1, the first human infections were with a virus that had only 15% of the mutations needed for the epidemic.

How and where did the virus learn to infect humans?

In the SARS1 epidemic, once an antibody test was available to identify people who had been infected, stored blood samples were tested for previous, undiagnosed infections. Workers in the market had a 20% positivity while in the general population you could find about 1% of people who were infected. These people were the training ground on which SARS1 learned to infect humans and learn to support human-to-human spread.

Did we find a similar training ground in stored human specimens from Wuhan before the pandemic?

No. 36,000 blood specimens were tested, and none were positive. If SARS2 was like SARS1 we would have expected at least 360 positive stored specimens.

Where could SARS2 have learned to infect human?

Replicating a virus in human cell cultures, that is, a test tube would do it. So would passing the virus in mice genetically modified to contain human lung tissue, so-called humanized mice. And we know that a US coronavirus researcher, funded by the NIH, provided the WIV with his laboratory’s humanized mice for doing this type of research.

The other SARS2 feature that contributed to infectivity and pathogenicity was the furin cleavage site. Many viruses use a host cell enzyme or scissors to cut a virus cell surface attachment protein as the last step before infectivity. And designing a virus in the laboratory that uses the enzyme furin by putting a synthetic furin cleavage site is a common go-to gain-of-function exercise. In fact, since 1992, at least 14 publications have described adding a furin cleavage site to a virus that didn’t have one, including a study from the WIV. 14 out of 14 times it makes the viruses nastier.

In fact, a grant application from 2018 involving a collaboration between US and Chinese scientists proposed synthetically inserting a “human specific furin cleavage site” into a bat virus. Finding the exact furin cleavage site in SARS2 that is also found naturally in an important human lung protein, ENAC, that controls water flow into the lung, is very suspicious. And the backbone of SARS2, about 96% of the virus, is identical to several bat viruses. Interestingly, SARS2 is so adapted to the human host that it can no longer infect bat cells in culture.

The SARS2 furin cleavage site is also unusual in two ways. In the 1000 years since the SARS2-related viruses separated from the other related beta coronaviruses, there has never been a virus with a furin cleavage site. It is also unusual in that it uses a rare genetic sequence that has also never been used by these related viruses in nature. The sequence is a common genetic sequence used in the lab when gene jocks juice up viruses.
The furin cleavage site in SARS2 also explains why this virus but not SARS1 can infect the brain, heart, lungs, kidneys, and other organs. This is because these organs also have the furin enzyme on their surface.

**What about gain-of-function features related to asymptomatic spread and immune system evasion?**

Since these two features would greatly increase the pandemic potential of a virus, governments and academic scientists have agreed not to conduct gain-of-function research in these areas.

**Where does SARS2 come in?**

SARS2 contains a protein called ORF8, so named because it is the eighth protein in the SARS2 genome. It is one of the only proteins that is not part of the finished virus or is involved in taking over the cellular machinery that makes new viruses. ORF8 is diabolical. It is made early after an infection before other viral proteins begin to be synthesized. At this point the cell is largely unaware it is infected and hasn’t mounted any defenses. ORF8 enters the blood stream and interacts with the immune system, doing two things.

First, it blocks the production of interferon. Interferon has two important functions:

First, it is a blunt weapon against infections that is used early by the body to slow down an infection, allowing time for antibodies to be produced and T-cells to respond. And second, it produces the familiar symptoms of an infection: fever, chills, sweating, red skin. The symptoms of an infection are not directly from the microbe itself but are from the body’s response to the presence of a microbe. Take interferon away and you have asymptomatic spread. I am aware of no other new respiratory virus that is asymptomatic when it first entered the human population. If you remember back to the early days of COVID, no one thought we could be missing infections because of lack of symptoms. We now know that 40% of COVID from the beginning was asymptomatic.

The other property of ORF8 is that it interferes with the immune system’s process of making antibodies and teaching T-cells about the virus. This so-called MHC antigen presentation system is important for fighting infections. The AIDS virus is the poster child of viruses that become chronic infections because, among other things, it inhibits the normal immune system response. No one knew about ORF8 and these features when the vaccine target was being selected and so immunity from vaccination does not include inhibiting ORF8. Interestingly, in a natural infection your body recognizes ORF8 as a highly foreign protein and actually makes more antibodies against it than any other protein.

**What does this have to do with gain-of-function research at the WIV?**

Prior to 2019, the WIV had conducted extensive research on optimizing the ORF8 gene and its function and on creating a synthetic biology pathway for manipulating this protein and putting it in viruses in the laboratory. This work was found in two master theses from students at the WIV that were never translated from Mandarin nor did they ever lead to publications. They were in fact found online by a group of amateur investigators self-named DRASTIC. I have found no western virologist that was doing research on ORF8 before the pandemic.
Knowing from the beginning that SARS2 had these three genetic features, that is, an optimized receptor binding domain, the effects of the furin cleavage site on transmissibility and multi-organ affinity, and the properties of ORF8 would have significantly helped in reducing the pandemics impact for three reasons:

- Human-to-human spread was accruing from the beginning and did not have to be acquired slowly, like with SARS1. The world lost almost a month of response time while public health officials made pronouncements about lack of human-to-human spread;
- Rapid spread within the body because of the humanized furin cleavage site, beginning in the lungs but leading often to multi-organ attack, could have guided treatment to better outcomes;
- And finally, knowing that 40% of cases were asymptomatic and that vaccines might be improved by including immunizing against ORF8 could have been easily done and might have improved vaccine efficacy, as well as not missing early asymptomatic cases.

Has gain-of-function research been useful to the COVID19 response or other public health infectious disease emergencies?

In looking at the collected gain-of-function research over approximately two decades, I have found no findings that could reasonably be considered to have helped in either the COVID pandemic or other smaller epidemics. At this point we know that an mRNA vaccine can be designed within literally days of a new outbreak once the pathogen is sequenced, and large-scale manufacturing can begin soon thereafter. This capability has now been fully road tested and provides, in my opinion, the best defensive capability against future microbes.

It is also important to point out that gain-of-function research is a tiny sliver of all the research funded by NIH. Specifically, there were over 36,000 RO1 grants funded by NIH in 2020, the latest year with statistics. Of these, the self-described "gain-of-function on potential pathogens" research grants numbered only twenty-one in the latest funding year. Even expanding this by tenfold with a less stringent definition of gain-of-function would mean we are talking about less than 1% of all NIH research funding.

I cannot imagine a scenario where, but for this tiny research effort, a new pandemic occurs.

What reforms should be considered in order to assure that such research is conducted in a safe and transparent manner?

While I find no actual benefit of gain-of-function research, I believe efforts to ban it, given the vested interests of literally the entire virology community, and maybe others, is a hill too steep to climb. A proposal that I believe is achievable is the placement of all select agent research within the existing institutional review board structure used for human clinical trials. The requirement to explain the cost-benefit of a particular set of experiments to unaffiliated lay people and community members could place guardrails on the field and eliminate the most dangerous research. The argument that the research is too complex to explain to non-scientists fails when you point out that chimeric antigen receptor genetic engineering of the human immune system to fight cancer is routinely placed within the oversight of the IRB system. This research is arguably more complex than gain-of-function work. In addition, the IRB system is an international standard that is used everywhere, including in China.
My second reform is to separate the governmental oversight of this research from the funding agency. We have now documented the failure of the internal NIH system, the so-called P3CO, to provide adequate oversight. The model is atomic energy research, which is funded by the Department of Defense, but which is overseen by the Atomic Energy Commission.

My third suggestion is to place the western technology of biotechnology under export controls and monitoring. There are ways to build into these systems a forensic and law enforcement capability that could, for example, with probably cause and a court-issued warrant allow

**What happens if we have these hearings, and nothing changes?**

A significant part of my prepared statement is derived from the evidence that I and others have collected related to the pandemic that can be sourced from outside of the WIV. In a court of law much of this would be called circumstantial evidence. While sometimes circumstantial evidence is characterized as of inferior quality it certainly does not need to be. Murders can be solved when the weapon or even the victim cannot be found. Convictions based solely on such circumstantial evidence happen all the time. But my final statement is based on actual evidence from inside the WIV. Please show the next chart.

In December 2019 five bronchial lavage specimens were taken from patients in Wuhan and sent to the WIV for analysis. The patients had pneumonia and using a sequencing machine from a US company was used to identify SARS2. The paper that was written about these patients was quickly published the first week of February and this paper has been viewed millions of times. The WIV also published the raw data that came from the specimen as well. These samples were massively expanded, using a PCR like process, and ultimately yielded tens of millions of reads of genetic material.

We took these specimen reads and conducted a forensic analysis, making three observations:

- **First, we confirmed they contained the SARS2 virus.**

- **Second, we identified 20 unexpected contaminants in the specimens that we suspected to be the inadvertent amplification of other research going on in the laboratory. Things not expected to be found in a human specimen like honey suckle genes or a horse virus. For 19 of the 20 unexpected contaminants, we then found published research from the previous two years, confirming that the lab had indeed been working on these unexpected genes. This also validated that our methods could detect covert research efforts.**

- **One contaminant was not accounted for in published papers. The chart shows this finding: a portion of the Nipah virus genome in a laboratory vector commonly used for synthetic biology.**

The Nipah virus is a smaller virus than SARS2 and is much less transmissible. But it is one of the deadliest viruses, with a >60% lethality. This is 60-times deadlier than SARS2. The lab where the human specimens were processed is not the highest level biosafety lab, BSL-4, but was in the BSL-2 or -3 facility.
Why were they conducting synthetic biology research in December 2019 on the Nipah virus? I cannot speculate.

But a laboratory-acquired infection with a modified Nipah virus would make the COVID19 pandemic look like a walk in the park.

The work of this committee is critical to protecting the American people and, really, the world at large, from future manmade pandemics. Thank you for the opportunity to speak before this committee.