The Freedom of Information Act (5 USC 552)

FOIA Exemptions

(b)(1) Information specifically authorized by an executive order to be kept secret in the interest of national defense or foreign policy. Executive Order 13526 includes the following classification categories:

1.4(a) Military plans, systems, or operations
1.4(b) Foreign government information
1.4(c) Intelligence activities, sources or methods, or cryptology
1.4(d) Foreign relations or foreign activities of the US, including confidential sources
1.4(e) Scientific, technological, or economic matters relating to national security, including defense against transnational terrorism
1.4(f) U.S. Government programs for safeguarding nuclear materials or facilities
1.4(g) Vulnerabilities or capabilities of systems, installations, infrastructures, projects, plans, or protection services relating to US national security, including defense against transnational terrorism
1.4(h) Weapons of mass destruction

(b)(2) Related solely to the internal personnel rules and practices of an agency

(b)(3) Specifically exempted from disclosure by statute (other than 5 USC 552), for example:

ARMSEXP	Arms Export Control Act, 50a USC 2411(c)
CIA PERS/ORG	Central Intelligence Agency Act of 1949, 50 USC 403(g)
EXPORT CONTROL	Export Administration Act of 1979, 50 USC App. Sec. 2411(c)
FS ACT	Foreign Service Act of 1980, 22 USC 4004
INA	Immigration and Nationality Act, 8 USC 1202(f), Sec. 222(f)
IRAN	Iran Claims Settlement Act, Public Law 99-99, Sec. 505

(b)(4) Trade secrets and confidential commercial or financial information

(b)(5) Interagency or intra-agency communications forming part of the deliberative process, attorney-client privilege, or attorney work product

(b)(6) Personal privacy information

(b)(7) Law enforcement information whose disclosure would:
(A) interfere with enforcement proceedings
(B) deprive a person of a fair trial
(C) constitute an unwarranted invasion of personal privacy
(D) disclose confidential sources
(E) disclose investigation techniques
(F) endanger life or physical safety of an individual

(b)(8) Prepared by or for a government agency regulating or supervising financial institutions

(b)(9) Geological and geophysical information and data, including maps, concerning wells

Other Grounds for Withholding

NR Material not responsive to a FOIA request excised with the agreement of the requester

From: (b)(6) @state.gov>
To: Paulopol, Andreea I (b)(6) @state.gov>; (b)(6) D@state.gov>; Gibbs, Jeffrey J (b)(6) @state.gov>; (b)(6) (b)(6) @state.gov>; (b)(6) @state.gov>; Gross, Laura J (b)(6) @state.gov>
CC: (b)(6) state.gov>; (b)(6) @state.gov>
Subject: RE: Nature Medicine re COVID19
Date: Mon, 14 Dec 2020 17:29:08 +0000

The Nature commentary piece is interesting, but I don't know to what extent Nature Correspondence is peer reviewed. There is no mention of peer review in the acknowledgements, nor anywhere else I could find. More importantly, it's also dated 17 March and has not been updated. I believe this was before the pre-outbreak serum studies showed no evidence of pre-outbreak mutation, let alone presence, of SARS-CoV-2. It was also probably too early for them to have observed that SARS-CoV-2 has not mutated at a rate they would expect from a zoonotic spillover based on previous SARS and MIRS spillover events.

It has been eight months since this Nature piece, a year since the first reported cases, and there still has been no candidate zoonotic host found. It would be good to know what represents the most recent, most substantive, and peer reviewed, evaluation of lab vs non-lab hypotheses.

From: Paulopol, Andreea I (b)(6) @state.gov>
Sent: Monday, December 14, 2020 11:04 AM
To: (b)(6) @state.gov>; Gibbs, Jeffrey J (b)(6) @state.gov>; (b)(6) (b)(6) state.gov>; (b)(6) @state.gov>; (b)(6) (b)(6) @state.gov>; Gross, Laura J (b)(6) @state.gov>
Cc: (b)(6) @state.gov>; (b)(6) @state.gov>
Subject: RE: (b)(6) summary of post cables/Nature Medicine re COVID19

I understand that some folks here may not be interested in the science, but the attached article from Nature Medicine may be worth reviewing.

Also, https://sciencebusiness.net/covid-19/international-news/china-was-slammed-initial-covid-19-secrecy-its-scientists-led-way

China was slammed for initial COVID-19 secrecy, but its scientists led the way in tackling the virus

"The Chinese have been leading the way in publishing open-access evidence on case management, genomics and numerous areas of public health and epidemiology, which has been vital in informing the response in more or less every country."

From: (b)(6) @state.gov>
Sent: Sunday, December 13, 2020 6:30 PM
To: Gibbs, Jeffrey J (b)(6) @state.gov>; (b)(6) @state.gov>; (b)(6) @state.gov>; (b)(6) @state.gov>; (b)(6) @state.gov>; Paulopol, Andreea I (b)(6) @state.gov>; Gross, Laura J (b)(6) @state.gov>
Cc: (b)(6) @state.gov>; (b)(6) @state.gov>
Subject: Re summary of post cables

Many of you may have read this article but it remains important as reminder of several inconvenient facts. Don't underestimate cognitive dissonance and wishful disbelief in science and government. The level of groupthink on the COVID19 origins issue is ridiculous. I personally pray it came straight out of Mother Nature but, thus far, have found no objective scientific analysis to explain it. So where did it come from—how, who, and why? The VCAWG should be a straight forward evidence based discussion, not supposition, imposition, or borderline superstition level theories.

https://www.bostonmagazine.com/news/2020/09/09/alina-chan-broad-institute-coronavirus/

RESEARCH

Could COVID-19 Have Escaped from a Lab?

The world's preeminent scientists say a theory from the Broad Institute's Alina Chan is too wild to be believed. But when the theory is about the possibility of COVID being man-made, is this science or censorship?

by **ROWAN JACOBSEN**, *9/9/2020, 9:29 a.m.*

- PRINT

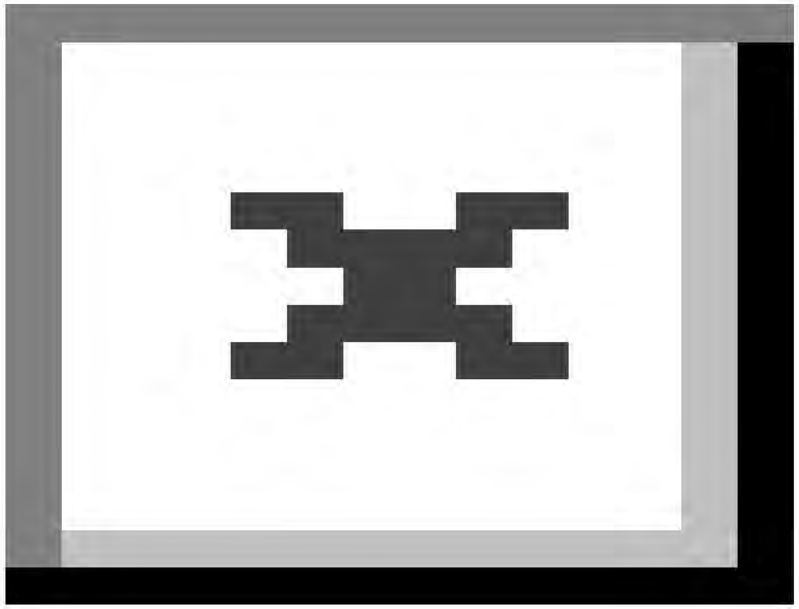


Illustration by Benjamen Purvis

In January, as she watched the news about a novel virus spreading out of control in China, Alina Chan braced for a shutdown. The molecular biologist at the Broad Institute of Harvard and MIT started stockpiling medicine and supplies. By the time March rolled around and a quarantine seemed imminent, she'd bought hundreds of dollars' worth of fillets from her favorite fishmonger in Cambridge and packed them into her freezer. Then she began to ramp down her

projects in the lab, isolating her experimental cells from their cultures and freezing them in small tubes.

As prepared as she was for the shutdown, though, she found herself unprepared for the frustration of being frozen out of work. She paced the walls of her tiny apartment feeling bored and useless. Chan has been a puzzle demon since childhood, which was precisely what she loved about her work—the chance to solve fiendishly difficult problems about how viruses operate and how, through gene therapy, they could be repurposed to help cure devastating genetic diseases. Staring out her window at the eerily quiet streets of her Inman Square neighborhood, she groaned at the thought that it could be months before she was at it again. Her mind wandered back to 2003, when she was a teenager growing up in Singapore and the first SARS virus, a close relative of this coronavirus, appeared in Asia. It hadn't been anything like this. That one had been relatively easy to corral. *How had this virus come out of nowhere and shut down the planet? Why was it so different?* she asked herself.

Then it hit her: The world's greatest puzzle was staring her in the face. Stuck at home, all she had to work with was her brain and her laptop. Maybe they were enough. Chan fired up the kettle for the first of what would become hundreds of cups of tea, stacked four boxes on her kitchen counter to raise her laptop to the proper height, pulled back her long dark hair, and began reading all of the scientific literature she could find on the coronavirus.

It wasn't long before she came across an article about the remarkable stability of the virus, whose genome had barely changed from the earliest human cases, despite trillions of replications. This perplexed Chan. Like many emerging infectious diseases, COVID-19 was thought to be zoonotic—it originated in animals, then somehow found its way into people. At the time, the Chinese government and most scientists insisted the jump had happened at Wuhan's seafood market, but that didn't make sense to Chan. If the virus had leapt from animals to humans in the market, it should have immediately started evolving to life inside its new human hosts. But it hadn't.

On a hunch, she decided to look at the literature on the 2003 SARS virus, which had jumped from civets to people. *Bingo*. A few papers mentioned its rapid evolution in its first months of existence. Chan felt the familiar surge of puzzle endorphins. The new virus really wasn't behaving like it should. Chan knew that delving further into this puzzle would require some deep genetic analysis, and she knew just the person for the task. She opened Google Chat and fired off a message to Shing Hei Zhan. He was an old friend from her days at the University of British Columbia and, more important, he was a computational god.

"Do you want to partner on a very unusual paper?" she wrote.

Sure, he replied.

One thing Chan noticed about the original SARS was that the virus in the first human cases was subtly different—a few dozen letters of genetic code—from the one in the civets. That meant it had immediately morphed. She asked Zhan to pull up the genomes for the coronaviruses that had

been found on surfaces in the Wuhan seafood market. Were they at all different from the earliest documented cases in humans?

Zhan ran the analysis. Nope, they were 100 percent the same. Definitely from humans, not animals. The seafood-market theory, which Chinese health officials and the World Health Organization espoused in the early days of the pandemic, was wrong. Chan's puzzle detectors pulsed again. "Shing," she messaged Zhan, "this paper is going to be insane."

In the coming weeks, as the spring sun chased shadows across her kitchen floor, Chan stood at her counter and pounded out her paper, barely pausing to eat or sleep. It was clear that the first SARS evolved rapidly during its first three months of existence, constantly fine-tuning its ability to infect humans, and settling down only during the later stages of the epidemic. In contrast, the new virus looked a lot more like late-stage SARS. "It's almost as if we're missing the early phase," Chan marveled to Zhan. Or, as she put it in their paper, as if "it was already well adapted for human transmission."

That was a profoundly provocative line. Chan was implying that the virus was already familiar with human physiology when it had its coming-out party in Wuhan in late 2019. If so, there were three possible explanations.

Perhaps it was just staggeringly bad luck: The mutations had all occurred in an earlier host species, and just happened to be the perfect genetic arrangement for an invasion of humanity. But that made no sense. Those mutations would have been disadvantageous in the old host.

Maybe the virus had been circulating undetected in humans for months, working out the kinks, and nobody had noticed. Also unlikely. China's health officials would not have missed it, and even if they had, they'd be able to go back now through stored samples to find the trail of earlier versions. And they weren't coming up with anything.

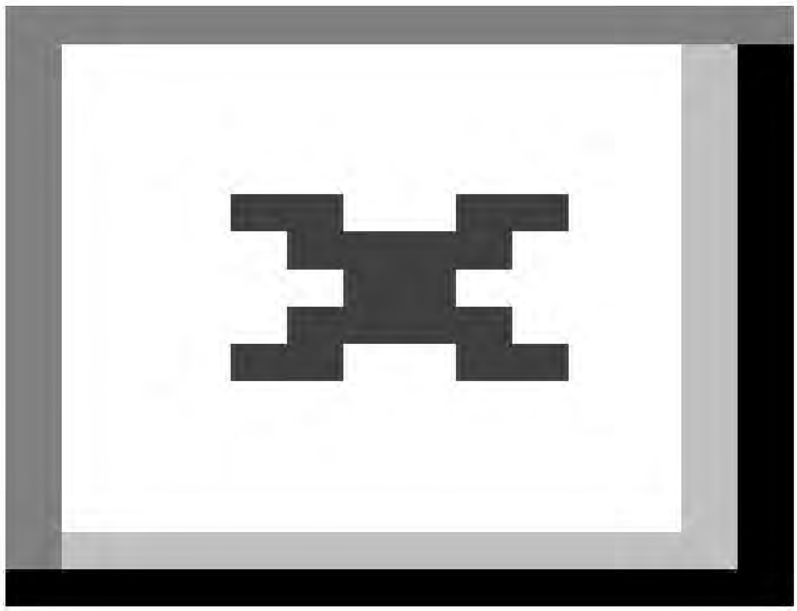
That left a third possibility: The missing phase had happened in a lab, where the virus had been trained on human cells. Chan knew this was the third rail of potential explanations. At the time, conspiracy theorists were spinning bioweapon fantasies, and Chan was loath to give them any ammunition. But she also didn't want to play politics by withholding her findings. Chan is in her early thirties, still at the start of her career, and an absolute idealist about the purity of the scientific process. Facts were facts.

Or at least they used to be. Since the start of the pandemic, the Trump administration has been criticized for playing fast and loose with facts—denying, exaggerating, or spinning them to suit the president's political needs. As a result, many scientists have learned to censor themselves for fear that their words will be misrepresented. Still, Chan thought, if she were to sit on scientific research just to avoid providing ammunition to conspiracy theorists or Trump, would she be any better than them?

Chan knew she had to move forward and make her findings public. In the final draft of her paper, she torpedoed the seafood-market theory, then laid out a case that the virus seemed curiously well adapted to humans. She mentioned all three possible explanations, carefully wording the third to emphasize that if the novel coronavirus did come from a lab, it would have been the result of an accident in the course of legitimate research.

On May 2, Chan uploaded the paper to a site where as-yet-unpublished biology papers known as "preprints" are shared for open peer review. She tweeted out the news and waited. On May 16, the *Daily Mail*, a British tabloid, picked up her research. The very next day, *Newsweek* ran a story with the headline "Scientists Shouldn't Rule Out Lab as Source of Coronavirus, New Study Says."

And that, Chan says, is when "shit exploded everywhere."



Alina Chan, a molecular biologist at the Broad Institute, says we can't rule out the possibility that the novel coronavirus originated in a lab—even though she knows it's a politically radioactive thing to say. / Photo by Mona Miri

Chan had come to my attention a week before the *Newsweek* story was published through her smart and straightforward tweets, which I found refreshing at a time when most scientists were avoiding any serious discussion about the possibility that COVID-19 had escaped from a biolab.

I'd written a lot about genetic engineering and so-called gain-of-function research—the fascinating, if scary, line of science in which scientists alter viruses to make them more transmissible or lethal as a way of assessing how close those viruses are to causing pandemics. I also knew that deadly pathogens escape from biolabs with surprising frequency. Most of these accidents end up being harmless, but many researchers have been infected, and people have died as a result.

For years, concerned scientists have warned that this type of pathogen research was going to trigger a pandemic. Foremost among them was Harvard epidemiologist Marc Lipsitch, who founded the Cambridge Working Group in 2014 to lobby against these experiments. In a series of policy papers, op-eds, and scientific forums, he pointed out that accidents involving deadly pathogens occurred more than twice a week in U.S. labs, and estimated that just 10 labs performing gain-of-function research over a 10-year period would run a nearly 20 percent risk of an accidental release. In 2018, he argued that such a release could "lead to global spread of a virulent virus, a biosafety incident on a scale never before seen."

Thanks in part to the Cambridge Working Group, the federal government briefly instituted a moratorium on such research. By 2017, however, the ban was lifted and U.S. labs were at it again. Today, in the United States and across the globe, there are dozens of labs conducting experiments on a daily basis with the deadliest known pathogens. One of them is the Wuhan Institute of Virology. For more than a decade, its scientists have been discovering coronaviruses in bats in southern China and bringing them back to their lab in Wuhan. There, they mix genes from different strains of these novel viruses to test their infectivity in human cells and lab animals.

When word spread in January that a novel coronavirus had caused an outbreak in Wuhan—which is a thousand miles from where the bats that carry this lineage of viruses are naturally found—many experts were quietly alarmed. There was no proof that the lab was the source of the virus, but the pieces fit.

Despite the evidence, the scientific community quickly dismissed the idea. Peter Daszak, president of EcoHealth Alliance, which has funded the work of the Wuhan Institute of Virology and other labs searching for new viruses, called the notion "preposterous," and many other experts echoed that sentiment.

That wasn't necessarily what every scientist thought in private, though. "They can't speak directly," one scientist told me confidentially, referring to the virology community's fear of having their comments sensationalized in today's politically charged environment. "Many virologists don't want to be hated by everyone in the field."

There are other potential reasons for the pushback. There's long been a sense that if the public and politicians really knew about the dangerous pathogen research being conducted in many laboratories, they'd be outraged. Denying the possibility of a catastrophic incident like this, then, could be seen as a form of career preservation. "For the substantial subset of virologists who perform gain-of-function research," Richard Ebright, a Rutgers microbiologist and another founding member of the Cambridge Working Group, told me, "avoiding restrictions on research funding, avoiding implementation of appropriate biosafety standards, and avoiding

implementation of appropriate research oversight are powerful motivators." Antonio Regalado, biomedicine editor of *MIT Technology Review*, put it more bluntly. If it turned out COVID-19 came from a lab, he tweeted, "it would shatter the scientific edifice top to bottom."

That's a pretty good incentive to simply dismiss the whole hypothesis, but it quickly amounted to a global gaslighting of the media—and, by proxy, the public. An unhealthy absolutism set in: Either you insisted that any questions about lab involvement were absurd, or you were a tool of the Trump administration and its desperation to blame China for the virus. I was used to social media pundits ignoring inconvenient or politically toxic facts, but I'd never expected to see that from some of our best scientists.

Which is why Chan stood out on Twitter, daring to speak truth to power. "It is very difficult to do research when one hypothesis has been negatively cast as a conspiracy theory," she wrote. Then she offered some earnest advice to researchers, suggesting that most viral research should be done with neutered viruses that have had their replicating machinery removed in advance, so that even if they escaped confinement, they would be incapable of making copies of themselves. "When these precautions are not followed, risk of lab escape is exponentially higher," she explained, adding, "I hope the pandemic motivates local ethics and biosafety committees to think carefully about how they can reduce risk." She elaborated on this in another tweet several days later: "I'd also—personally—prefer if high biosafety level labs were not located in the most populous cities on earth."

How Safe Are Boston's Biolabs?

As one of the world centers of biotech, the Hub is peppered with academic and corporate labs doing research on pathogens. Foremost among them is Boston University's National Emerging Infectious Diseases Laboratories (NEIDL), the only lab in the city designated as BSL-4 (the highest level of biosafety and the same level as the Wuhan Institute of Virology). It is one of just a dozen or so in the United States equipped to work with live versions of the world's most dangerous viruses, including Ebola and Marburg. Researchers there began doing so in 2018 after a decade of controversy: Many locals objected to the risks of siting such a facility in the center of a major metropolitan area.

The good news? Before opening, NEIDL undertook one of the most thorough risk assessments in history, learning from the mistakes of other facilities. Even Lynn Klotz, a senior science fellow at the Washington, DC–based Center for Arms Control and Non-Proliferation, who advised local groups that opposed NEIDL, told the medical website Contagion that the lab likely has the best possible security protocols and measures in place.

But the reality, Klotz added, is that most lab accidents are caused by human error, and there is only so much that can be done through good design and protocols to proactively prevent such mistakes. (Or to guard against an intentional release by a disgruntled researcher, as allegedly happened in the anthrax attacks of 2001.) Rutgers molecular biologist Richard Ebright, a longtime critic of potentially dangerous pathogen research, says the risks introduced by NEIDL are not low enough and "definitely not" worth the negligible benefits.

Still, risk is relative. Klotz has estimated the chance of a pathogen escape from a BSL-4 lab at 0.3 percent per year, and NEIDL is probably significantly safer than the typical BSL-4 lab. And if catching a deadly pathogen is your fear, well, currently you run a good risk of finding one in your own neighborhood. Until that gets cleared up, the city's biolabs are probably among the safer spaces in town.

Chan had started using her Twitter account this intensely only a few days earlier, as a form of outreach for her paper. The social platform has become the way many scientists find out about one another's work, and studies have shown that attention on Twitter translates to increased citations for a paper in scientific literature. But it's a famously raw forum. Many scientists are

not prepared for the digital storms that roil the Twitterverse, and they don't handle it well. Chan dreaded it at first, but quickly took to Twitter like a digital native. "Having Twitter elevates your work," she says. "And I think it's really fun to talk to nonscientists about that work."

After reading her tweets, I reviewed her preprint, which I found mind-blowing, and wrote her to say so. She thanked me and joked that she worried it might be "career suicide."

It wasn't long before it began to look like she might be right.

Speaking her mind, it turns out—even in the face of censure—was nothing new for Chan, who is Canadian but was raised in Singapore, one of the more repressive regimes on earth. Her parents, both computer science professionals, encouraged free thinking and earnest inquiry in their daughter, but the local school system did not. Instead, it was a pressure-cooker of a system that rewarded students for falling in line, and moved quickly to silence rebels.

That was a bad fit for Chan. "You have to bow to teachers," she says. "Sometimes teachers from other classes would show up and ask me to bow to them. And I would say, 'No, you're not my teacher.' Back then they believed in corporal punishment. A teacher could just take a big stick and beat you in front of the class. I got whacked so many times."

Still, Chan rebelled in small ways, skipping school and hanging out at the arcade. She also lost interest in her studies. "I just really didn't like school. And I didn't like all the extracurriculars they pack you with in Singapore," she says. That changed when a teacher recruited her for math Olympiads, in which teams of students compete to solve devilishly hard arithmetic puzzles. "I really loved it," she says. "You just sit in a room and think about problems."

Chan might well have pursued a career in math, but then she came up against teams from China in Olympiad competitions. "They would just wipe everyone else off the board," she says. "They were machines. They'd been trained in math since they could walk. They'd hit the buzzer before you could even comprehend the question. I thought, *I'm not going to survive in this field.*"

Chan decided to pursue biology instead, studying at the University of British Columbia. "I liked viruses from the time I was a teen," she says. "I remember the first time I learned about HIV. I thought it was a puzzle and a challenge." That instinct took her to Harvard Medical School as a postdoc, where the puzzle became how to build virus-like biomolecules to accomplish tasks inside cells, and then to Ben Deverman's lab at the Broad Institute. "When I see an interesting question, I want to spend 100 percent of my time working on it," she says. "I get really fixated on answering scientific questions."

Deverman, for his part, says he wasn't actively looking to expand his team when Chan came along, but when "opportunities to hire extraordinary people fall in my lap," he takes them. "Alina brings a ton of value to the lab," he explains, adding that she has an ability to pivot between different topics and cut to the chase. Nowhere was that more on display than with her coronavirus work, which Deverman was able to closely observe. In fact, Chan ran so many ideas past him that he eventually became a coauthor. "She is insightful, determined, and has the rare ability to explain complex scientific findings to other scientists and to the public," he says.

Those skills would prove highly useful when word got out about her coronavirus paper.

If Chan had spent a lifetime learning how to pursue scientific questions, she spent most of the shutdown learning what happens when the answers you come up with are politically radioactive. After the *Newsweek* story ran, conservative-leaning publications seized on her paper as conclusive evidence that the virus had come from a lab. "Everyone focused on the one line," Chan laments. "The tabloids just zoomed in on it." Meanwhile, conspiracists took it as hard evidence of their wild theories that there had been an intentional leak.

Chan spent several exhausting days putting out online fires with the many people who had misconstrued her findings. "I was so naive," she tells me with a quick, self-deprecating laugh. "I just thought, *Shouldn't the world be thinking about this fairly?* I really have to kick myself now."

Even more troubling, though, were the reactions from other scientists. As soon as her paper got picked up by the media, luminaries in the field sought to censure her. Jonathan Eisen, a well-known professor at UC Davis, criticized the study in *Newsweek* and on his influential Twitter account, writing, "Personally, I do not find the analysis in this new paper remotely convincing." In a long thread, he argued that comparing the new virus to SARS was not enough to show that it was preadapted to humans. He wanted to see comparisons to the initial leap of other viruses from animals to humans.

Moments later, Daszak piled on. The NIH had recently cut its grant to his organization, EcoHealth Alliance, after the Trump administration learned that some of it had gone to fund the Wuhan Institute of Virology's work. Daszak was working hard to get it restored and trying to stamp out any suggestion of a lab connection. He didn't hold back on Chan. "This is sloppy research," he tweeted, calling it "a poorly designed phylogenetic study with too many inferences and not enough data, riding on a wave of conspiracy to drive a higher impact." Peppering his tweets with exclamation points, he attacked the wording of the paper, arguing that one experiment it cited was impossible, and told Chan she didn't understand her own data. Afterward, a Daszak supporter followed up his thread with a GIF of a mike drop.

It was an old and familiar dynamic: threatened silverback male attempts to bully a junior female member of the tribe. As a postdoc, Chan was in a vulnerable position. The world of science is still a bit medieval in its power structure, with a handful of institutions and individuals deciding who gets published, who gets positions, who gets grants. There's little room for rebels.

What happened next was neither old nor familiar: Chan didn't back down. "Sorry to disrupt mike drop," she tweeted, providing a link to a paper in the prestigious journal *Nature* that "does that exact experiment you thought was impossible." Politely but firmly, she justified each point Daszak had attacked, showing him his mistakes. In the end, Daszak was reduced to arguing that she had used the word "isolate" incorrectly. In a coup de grâce, Chan pointed out that actually the word had come from online data provided by GenBank, the NIH's genetic sequence database. She offered to change it to whatever made sense. At that point, Daszak stopped replying. He insists, however, that Chan is overinterpreting her findings.

With Eisen, Chan readily agreed to test her hypothesis by finding other examples of viruses infecting new hosts. Within days, a perfect opportunity came along when news broke that the coronavirus had jumped from humans to minks at European fur farms. Sure enough, the mink

version began to rapidly mutate. "You actually see the rapid evolution happening," Chan said. "Just in the first few weeks, the changes are quite drastic."

Chan also pointed out to Eisen that the whole goal of a website such as bioRxiv (pronounced "bioarchive")—where she posted the paper—is to elicit feedback that will make papers better before publication. Good point, he replied. Eventually he conceded that there was "a lot of interesting analysis in the paper" and agreed to work with Chan on the next draft.

The Twitter duels with her powerful colleagues didn't rattle Chan. "I thought Jonathan was very reasonable," she says. "I really appreciated his expertise, even if he disagreed with me. I like that kind of feedback. It helped to make our paper better."

With Daszak, Chan is more circumspect. "Some people have trouble keeping their emotions in check," she says. "Whenever I saw his comments, I'd just think, *Is there something I can learn here? Is there something he's right about that I should be fixing?*" Ultimately, she decided, there was not.

By late May, both journalists and armchair detectives interested in the mystery of the coronavirus were discovering Chan as a kind of Holmes to our Watson. She crunched information at twice our speed, zeroing in on small details we'd overlooked, and became a go-to for anyone looking for spin-free explications of the latest science on COVID-19. It was thrilling to see her reasoning in real time, a reminder of why I've always loved science, with its pursuit of patterns that sometimes leads to exciting revelations. The website CNET featured her in a story about "a league of scientists-turned-detectives" who were using genetic sequencing technologies to uncover COVID-19's origins. After it came out, Chan added "scientist-turned-detective" to her Twitter bio.

She's lived up to her new nom de tweet. As the search for the source of the virus continued, several scientific teams published papers identifying a closely related coronavirus in pangolins—anteater-like animals that are heavily trafficked in Asia for their meat and scales. The number of different studies made it seem as though this virus was ubiquitous in pangolins. Many scientists eagerly embraced the notion that the animals might have been the intermediate hosts that had passed the novel coronavirus to humans. It fit their preexisting theories about wet markets, and it would have meant no lab had been involved.

As Chan read the pangolin papers, she grew suspicious. The first one was by a team that had analyzed a group of the animals intercepted by anti-smuggling authorities in southern China. They found the closely related virus in a few of them, and published the genomes for that virus. Some of the other papers, though, were strangely ambiguous about where their data was coming from, or how their genomes had been constructed. Had they really taken samples from actual pangolins?

Once again, Chan messaged Shing Hei Zhan. "Shing, something's weird here," she wrote. Zhan pulled up the raw data from the papers and compared the genomes they had published. Individual copies of a virus coming from different animals should have small differences, just as individuals of a species have genetic differences. Yet the genomes in all of the pangolin papers were perfect matches—the authors were all simply using the first group's data set. Far from being ubiquitous,

the virus had been found only in a few pangolins who were held together, and it was unclear where they had caught it. The animals might have even caught it from their own smuggler.

Remarkably, one group of authors in *Nature* even appeared to use the same genetic sequences from the other paper as if it were confirmation of their own discovery. "These sequences appear to be from the same virus (Pangolin-CoV) that we identified in the present study."

Chan called them out on Twitter: "Of course it's the same Pangolin-CoV, you used the same dataset!" For context, she later added, "Imagine if clinical trials were playing fast and loose with their patient data; renaming patients, throwing them into different datasets without clarification, possibly even describing the same patient multiple times across different studies unintentionally."

She and Zhan posted a new preprint on bioRxiv dismantling the pangolin papers. Confirmation came in June when the results of a study of hundreds of pangolins in the wildlife trade were announced: Not a single pangolin had any sign of a coronavirus. Chan took a victory lap on Twitter: "Supports our hypothesis all this time." The pangolin theory collapsed.

Chan then turned her Holmesian powers on bigger game: Daszak and the Wuhan Institute of Virology. Daszak had been pleading his case everywhere from *60 Minutes* to the *New York Times* and has been successful in rallying sympathy to his cause, even getting 77 Nobel laureates to sign a letter calling for the NIH to restore EcoHealth Alliance's funding.

In several long and detailed "tweetorials," Chan began to cast a cloud of suspicion on the WIV's work. She pointed out that scientists there had discovered a virus that is more than 96 percent identical to the COVID-19 coronavirus in 2013 in a mineshaft soon after three miners working there had died from a COVID-like illness. The WIV didn't share these findings until 2020, even though the goal of such work, Chan pointed out, was supposedly to identify viruses with the potential to cause human illnesses and warn the world about them.

Even though that virus had killed three miners, Daszak said it wasn't considered a priority to study at the time. "We were looking for SARS-related virus, and this one was 20 percent different. We thought it was interesting, but not high risk. So we didn't do anything about it and put it in the freezer," he told a reporter from *Wired*. It was only in 2020, he maintained, that they started looking into it once they realized its similarity to COVID-19. But Chan pointed to an online database showing that the WIV had been genetically sequencing the mine virus in 2017 and 2018, analyzing it in a way they had done in the past with other viruses in preparation for running experiments with them. Diplomatic yet deadpan, she wrote, "I think Daszak was misinformed."

For good measure, almost in passing, Chan pointed out a detail no one else had noticed: COVID-19 contains an uncommon genetic sequence that has been used by genetic engineers in the past to insert genes into coronaviruses without leaving a trace, and it falls at the exact point that would allow experimenters to swap out different genetic parts to change the infectivity. That same sequence can occur naturally in a coronavirus, so this was not irrefutable proof of an unnatural origin, Chan explained, "only an observation." Still, it was enough for one Twitter user to muse,

"If capital punishment were as painful as what Alina Chan is doing to Daszak/WIV regarding their story, it would be illegal."

Daszak says that indeed he had been misinformed and was unaware that that virus found in the mine shaft had been sequenced before 2020. He also says that a great lab, with great scientists, is now being picked apart to search for suspicious behavior to support a preconceived theory. "If you believe, deep down, something fishy went on, then what you do is you go through all the evidence and you try to look for things that support that belief," he says, adding, "That is not how you find the truth."

Many of the points in Chan's tweetorials had also been made by others, but she was the first reputable scientist to put it all together. That same week, London's *Sunday Times* and the BBC ran stories following the same trail of breadcrumbs that Chan had laid out to suggest that there had been a coverup at the WIV. The story soon circulated around the world. In the meantime, the WIV has steadfastly denied any viral leak. Lab director Yanyi Wang went on Chinese television and described such charges as "pure fabrication," and went on to explain that the bat coronavirus from 2013 was so different than COVID that it could not have evolved into it this quickly and that the lab only sequenced it and didn't obtain a live virus from it.

To this day, there is no definitive evidence as to whether the virus occurred naturally or had its origins in a lab, but the hypothesis that the Wuhan facility was the source is increasingly mainstream and the science behind it can no longer be ignored. And Chan is largely to thank for that.

In late spring, Chan walked through the tall glass doors of the Broad Institute for the first time in months. As she made her way across the gleaming marble foyer, her sneaker squeaks echoed in the silence. It was like the zombie apocalypse version of the Broad; all the bright lights but none of the people. It felt all the weirder that she was wearing her gym clothes to work.

A few days earlier, the Broad had begun letting researchers back into their labs to restart their projects. All computer work still needed to be done remotely, but bench scientists such as Chan could pop in just long enough to move along their cell cultures, provided they got tested for the virus every four days.

In her lab, Chan donned her white lab coat and took inventory, throwing out months of expired reagents and ordering new materials. Then she rescued a few samples from the freezer, took her seat at one of the tissue-culture hoods—stainless steel, air-controlled cabinets in which cell engineers do their work—and began reviving some of her old experiments.

She had mixed emotions about being back. It felt good to free her gene-therapy projects from their stasis, and she was even more excited about the new project she and Deverman were working on: an online tool that allows vaccine developers to track changes in the virus's genome by time, location, and other characteristics. "It came out of my personal frustration at not being able to get answers fast," she says.

On the other hand, she missed being all-consumed by her detective work. "I wanted to stop after the pangolin preprint," she says, "but this mystery keeps drawing me back in." So while she

waits for her cell cultures to grow, she's been sleuthing on the side—only this time she has more company: Increasingly, scientists have been quietly contacting her to share their own theories and papers about COVID-19's origins, forming something of a growing underground resistance. "There's a lot of curiosity," she says. "People are starting to think more deeply about it." And they have to, she says, if we are going to prevent future outbreaks: "It's really important to find out where this came from so it doesn't happen again."

That is what keeps Chan up at night—the possibility of new outbreaks in humans from the same source. If the virus emerged naturally from a bat cave, there could well be other strains in existence ready to spill over. If they are closely related, whatever vaccines we develop might work on them, too. But that might not be the case with manipulated viruses from a laboratory. "Someone could have been sampling viruses from different caves for a decade and just playing mix-and-match in the lab, and those viruses could be so different from one another that none of our vaccines will work on them," she says. Either way, "We need to find where this came from, and close it down."

Whatever important information she finds, we can be sure Chan will share it with the world. Far from being shaken by the controversy her paper stirred, she is more committed than ever to holding a line that could all too easily be overrun. "Scientists shouldn't be censoring themselves," she says. "We're obliged to put all the data out there. We shouldn't be deciding that it's better if the public doesn't know about this or that. If we start doing that, we lose credibility, and eventually we lose the public's trust. And that's not good for science." In fact, it would cause an epidemic of doubt, and that wouldn't be good for any of us.

From: Gibbs, Jeffrey J (b)(6) @state.gov>
Sent: Sunday, December 13, 2020 2:42 PM
To: (b)(6) @state.gov>; (b)(6) @state.gov>; (b)(6) (b)(6) @state.gov>; Paulopol, Andreea I (b)(6) @state.gov>; (b)(6) (b)(6) @state.gov>; Gross, Laura J (b)(6) @state.gov>
Cc: (b)(6) @state.gov>; (b)(6) @state.gov>
Subject: Re: summary of post cables

(b)(6)

Great. Points 2, 4, and 5 are key and form a basis for raising concerns about adherence to Article X at least, questions under Article V, and separately, possible concerns about compliance with Article V. I will to get a copy of the WHO regs as well.

Jeff Gibbs
Senior Adviser AVC
SSD/AVC
c: (b)(6)

From: (b)(6) @state.gov>
Sent: Saturday, December 12, 2020 12:43 PM

To: (b)(6) @state.gov>;(b)(6) @state.gov>; Paulopol, Andreea I(b)(6) @state.gov>;(b)(6) @state.gov>; Gross, Laura J (b)(6) @state.gov>
Cc:(b)(6) @state.gov>;(b)(6) @state.gov>; Gibbs, Jeffrey J (b)(6) @state.gov>;(b)(6) @state.gov>
Subject:(b)(6) summary of post cables

Thanks, (b)(6)

Inclined to think we can cite some of these in the CR as sources of concerns...

(BTW, Bullets 1, 4, and 5 (especially 5) have been widely reported on Taiwan TV.)

From:(b)(6) @state.gov>
Sent: Saturday, December 12, 2020 11:46 AM
To:(b)(6) @state.gov>; Paulopol, Andreea I(b)(6) @state.gov>;(b)(6) (b)(6) @state.gov>; Gross, Laura J(b)(6) @state.gov>
Cc:(b)(6) @state.gov>;(b)(6) @state.gov>;(b)(6) (b)(6) @state.gov>; Gibbs, Jeffrey J(b)(6) @state.gov>
Subject: Re: response from CDC/NIH

I have found over 90 Embassy Beijing cables dealing with COVID-19 from 7 Jan to present. As luck would have it, we had consulate personnel on the scene in Wuhan. I have harvested about half of the cables -- I am almost up to March. So far, these cables document:

- Gross corruption and ineptitude by the local government officials (some of whom were later fired). Arguably, these officials enabled COVID-19 to go from outbreak to epidemic to pandemic.
- Frequent requests from USG via multiple channels to try to assist, scientifically collaborate, and more importantly, attempt to obtain critical data on the epidemiological and medical aspects of the outbreak as it was spreading throughout China and to other countries.
- Private PPE and other donations from the US.
- Consistent stonewalling by the PRC as the epidemic grows into a global pandemic.
- The WHO publicly saying what the PRC wanted, and privately/unofficially/candidly complaining about the utter lack of transparency and cooperation.
    - Note: On multiple occasions WHO leaders publicly praised the PRC leadership for adhering to international health standards and aggressively attacking the virus.

Sender: (b)(6) @state.gov>
Recipient: Paulopol, Andreea I(b)(6) @state.gov>;

(b)(6) @state.gov>;
Gibbs, Jeffrey J (b)(6) @state.gov>;
(b)(6) @state.gov>;
(b)(6) @state.gov>;
Gross, Laura J (b)(6) @state.gov>;
(b)(6) @state.gov>;
(b)(6) @state.gov>

Would you like a little coronavirus with your sourdough bread?

Evidence for a laboratory-derived, gain-of-function modified coronavirus as the source for SARS-CoV-2

Steven Carl Quay, MD, PhD

Steven@DrQuay.com

ABSTRACT

The origin of the SARS-CoV-2 is not known. In this paper an analysis is conducted from a broader definition of zoonosis, namely: a disease that is harbored and transmitted from vertebrate nonhuman animals, or from *in vitro* cell cultures derived from vertebrate nonhuman animals, to humans. (The underlined portion is this author's revised definition).

Using this broader definition, eight lines of evidence provide support for the hypothesis that SARS-CoV-2 was a laboratory constructed, gain-of-function coronavirus that infected a facility staff member and then began the pandemic with a highly virulent human-to-human transmission.

In this scenario an intermediate host will never be found, unlike in the horseshoe bat for SARS or the camel for MERS, both true zoonotic spread. These lines of evidence are the following:

1. No seroconversion found in retained specimens (0/1723) from before January 2020.
2. No posterior diversity of background mutations during epidemic, which was seen with MERS. In the case of MERS, 93% of genomes had approximately 50 background SNPs that differed from the index case and also defined clades within the camel populations. With SARS-CoV-2 all human genomes "collapse back" into the index patient sequence, consistent with a laboratory-derived pure culture coronavirus at the beginning.
3. SARS and MERS, two previous natural-sourced diseases were, initially seen in humans in rural locations, where wildlife is located. Six previous laboratory-sourced cases of SARS were from the urban cities of Singapore, Beijing, and Taipei, where virology laboratories were located. The three hospitals where COVID-19 adult patients were admitted before Jan 2 2020 are located 2, 9, and 11 km from the Wuhan Institute of Virology. The approximate geo-center of the residences of five children with COVID-19 hospitalized during the first two weeks of January is the Wuhan Institute of Virology.
4. The Hunan Market is not the source of COVID-19. As has been said before, "it went into the market before it came out." The three hospitals where initial cases appeared lay along a path between the Market and the Virology Institute, which approximates Line 2 of the Wuhan Metro rapid transit subway system. This line was the first transit line to cross under the Yangtze River and has an average daily ridership of 1,000,000 people.
5. Phylogenetic analyses of relatedness of coronaviruses is based on a model of natural, in the wild, rates of mutation and recombination. For example, the success with attributing the SARS-CoV 2003 epidemic to a series of recombination events in horseshoe bats was the finding, in a single cave, of three bat coronaviruses that, with only a few recombinations, could produce the human virus. On the other hand, a hallmark of laboratory-created interspecies chimeric coronaviruses based on RNA recombination is the apparently incredible conclusions a phylogenetic analysis produces. For example, the murine-feline chimeric coronavirus that is used in a laboratory manual on methodologies has an estimated most recent common ancestor in 1647 ACE. Likewise, the analysis of the bat virus backbone for SARS-CoV-2 requires the recombination of bat sequences collected from bats in 12 of China's 26 provinces and with recombination dates 40 to 70 years in the past. The closest source of the Spike Protein gene, the pangolin, has a most recent common ancestor estimated at 1200 ACE. A parsimonious alternative would be a laboratory-generated virus.

6. The key to human infectivity of SARS-CoV-2, the furin cleavage site (FCS), is not found naturally in any lineage B coronavirus. But it has been introduced in coronaviruses via laboratory constructs all over the world, beginning in 2004, for gain-of-function experiments.
7. In the wild the synonymous codon usage bias (SCUB) of the entire coronavirus family is stable both over time and regardless of host species. In the laboratory, synonymous codon usage is changed at will and in fact changes are a standard practice, either to make a virus gene be expressed better by using "human optimized" codons for expression in mammalian cells or for the development of vaccines using viruses with identical protein coding but "de-optimized" codons, to slow expression and create an attenuated infection and thus a strong immune response. The furin cleavage site insertion of SARS-CoV-2 uses –CGG-CGG- for the RR dimer. In all coronaviruses, CCG is the least used of the six arginine codons and in one analysis is the 63rd out of 64th codon for overall usage. Coronavirus in the wild doesn't like CGG, let alone two of them together. On the other hand, CGG is a standard codon usage for arginine in many commercial "human optimized" kits for inserting genetic material. In an analysis that includes SARS-CoV-2, all other coronaviruses that infect humans, bat-CoV-RaTG13, and pangolin-CoV- _GX-P4L, (the two viruses said to be the closest in lineage to SARS-CoV-2) and that covered almost 300,000-nt, 139 RR dimers were found. The only –CGG-CGG- dimer was the insert in SARS-CoV-2, as would be expected for a laboratory-derived "codon optimized," construct. Various probabilities of a chance usage, based on in-the-wild SCUB are <0.001. Publications from laboratories in Wuhan describe the usage of CCG-containing primers for arginine codons.
8. An analysis of approximately 16,000 near-full length SARS-CoV-2 genomes on GISAID found that there was an approximately three-fold higher rate of synonymous SNP rate for the third nt of each CGG codon, changing it to an A or T and occurring within 60 days of the beginning of human spread. The significance of this is that, had SARS-CoV-2 been in circulation in the wild for any significant, it is likely purification of this unusual codon bias would have occurred.

Many of the lines of inquiry described herein have not been explored in depth, due to limitations of the author's access to bioinformatics and laboratory tools. It is hoped that each of these can be used as a spring board for the reexamination of the likely origin of this pandemic as well as to re-think the decision to end the ban on gain-of-function experiments.

While explaining to an educated, non-scientist a paper in which synthetic biology was used to engineer and resurrect chemically-synthetized clones of SARS-CoV-2 in only a week after receipt of the synthetic DNA fragments, all of this in baker's yeast, *Saccharomyces cerevisiae*, his unfortunate misunderstanding of the import of this research was to respond sarcastically, "So you're saying, would you like some coronavirus with that sourdough bread, is a real possibility?"

Clearly a public debate is needed.

INTRODUCTION

The COVID-19 pandemic, caused by the coronavirus SARS-CoV-2, has infected 4,009,472, with 275,914 deaths, as of 08 May 2020. From an early time, two theories of origin of the virus have be posited: a zoonotic event, in which the virus jumped from a reservoir animal to a human, who became "Patient Zero;" and a laboratory accident event, in which a laboratory-grown virus infected a laboratory facility employee, who became "Patient Zero."

On February 19, 2020 *The Lancet* published a Correspondence entitled "Statement in support of the scientists, public health professionals, and medical professionals of China combatting COVID-19[1]" with 27 public health scientists from eight countries as authors. The statement seems to attempt to settle the question of the origin of SARS-CoV-2 and short circuit further debate, as the second sentence reads: "We stand together to strongly condemn conspiracy theories suggesting that COVID-19 does not have a natural origin." It goes on to state: "Conspiracy theories do nothing but create fear, rumors, and prejudice that jeopardize our global collaboration in the fight against this virus."

The letter provided an open solicitation for support and at this time has been signed by at least 17,500 people, as if to purport that science can be advanced through the democratic process.[2] While it is a truism that conspiracy theories have no place in the academia, legitimate debate should not be foreclosed.

The statement itself provides a more nuanced discussion of the evidence for a zoonotic origin and contains 14 references, eight of which contain data about the COVID-19 pandemic and six of which are governmental policy statements without new data, background articles from 2003 and 2004 on zoonotic diseases, or a virus naming statement by the Coronavirus Study Group (CSG) of the International Committee on Taxonomy of Viruses, which is responsible for developing the official classification of viruses and taxa naming (taxonomy) of the Coronaviridae family. The eight articles with data were written at the end of January or early February, when there were fewer than 10,000 patients.

An analysis of the evidence for a zoonotic source given in support of the above Statement is contained in a Supplemental Material file attached to this manuscript. The analysis shows there was very little actual data available at the time to permit reaching such a definitive conclusion. There was also the absence of data or discussion that could support a laboratory origin.

For example, the 2004 report in *The Scientist* in which the WHO documented that the 2002 coronavirus, SARS, had escaped the Chinese Institute of Virology in two separate incidences, one via a postgraduate student and one via a postdoc, with subsequent community spread and even a death, was not offered. At the time, a WHO representative criticized the laboratory's safeguards.[3]

The purpose of this paper is to revisit the origin question in light of the totality of data available at this time, including the 17,682 genome sequence data curated by GISAID.[4]

---

[1] https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30418-9/fulltext#back-bib1

[2] This is reminiscent of the story attributed to Albert Einstein by Stephen Hawkins in his *Brief History of Time*. According to Hawkins, a book was published in 1930 in pre-war Germany entitled, "One Hundred Authors Against Einstein." When asked about the book Einstein retorted, "If I were wrong, then one would have been enough!"

[3] https://www.the-scientist.com/news-analysis/sars-escaped-beijing-lab-twice-50137

[4] https://www.gisaid.org/

RESULTS AND DISCUSSION

A new framework and broadened definition of zoonotic disease. While the use of the word zoonosis can be traced to the middle of the nineteenth century, a general agreement of its definition is: "a disease that is harbored and transmitted from vertebrate nonhuman animals to humans.[5]" This means that diseases communicated to humans from invertebrates, such as malaria, are excluded. For purposes of this discussion I would like to propose a broadening definition of zoonosis:

A disease that is harbored and transmitted from vertebrate nonhuman animals, **or from *in vitro* cell cultures derived from vertebrate nonhuman animals,** to humans.

When this definition of zoonosis is used, an accidental infection in a laboratory, the purchase of infected animals in a wet market, or the acquisition of human disease from domesticated camels are different examples of the same phenomenon, a rare event in which a human is infected from a stable non-human-microbial "reservoir" and then human-to-human transmission ensues. The following Text-Table provides the commonalities and distinguishing features of this broadened zoonosis definition, with the special cases for coronaviruses noted herein.

Pre-Human Transfer Source	Intermediate Reservoir Examples	Intermediate Reservoir Features That Increase Probability of the Rare Transfer Humans to Occur	Features which decrease probability of further "Patient Zero" human transfer	Examples	Human Phylogenic Features
Wildlife in nature	Bats and pangolin	Rural locations with high populations of wildlife.	Urbanization of natural habitat	Bats in caves	Posterior diversity into the reservoir
Wildlife in human food chain	Bats, civets, or racoon dogs	Cultures in which live, wildlife can be directly purchased by customers, butchered for food by workers, and cooked in local restrauants. Reports of the earliest cases of SARS in Guangdong involved employees of exotic meat markets.	Distance from wet market	Wet markets in China	Posterior diversity into the reservoir
Domesticated animals	Camels in MERS	Domestication in a rural or semi-rural location by workers in close contact	Distance from farms and markets	Markets with camels in the Middle East	Posterior diversity into the reservoir
Laboratory	VERO cells, an aneuploid, kidney epithelial cell culture extracted from an African green monkey	Extensive, daily contact with active microbe-host cell cultures; laboratories in which large numbers of specimens from nature are collected and curated	Distance from laboratory	Two "escapes" of the SARS 2002 coronavirus from a Bejing lab in 2004	Since there is no genetically diverse reservoir for continued jump into humans, there will be no posterior diversity into a genetically diverse reservoir.

A hallmark of a 'wildlife in nature' source is the finding of serological evidence for bat-borne coronavirus transmission to people. Such a study[6] was conducted (titled "Human-animal interactions and bat coronavirus spillover potential among rural residents in Southern China") and direct contact with bats was not identified as a risk factor. However, self-reported severe acute respiratory infection (SARI) and/or influenza-like illness (ILI) was linked to human interaction with other wildlife and livestock, suggesting that there may be other zoonotic exposures leading to clinical illness in these populations.

Evidence that urbanization diminishes a wildlife source theory by changes bat host range is abundant.[7]

In the future, geo-tracking can be used for contact trace analysis and efforts to find a non-laboratory source for epidemics. A study[8] entitled, "Explorative Analysis of Wuhan Intra-Urban Human Mobility Using Social Media Check-In Data," provides a framework for such investigation and might even be used in the current pandemic for a retrospective analysis if mobile phone records from fall 2019 remain within Wuhan telecommunication company records. The geo-tracking of the earliest cases of COVID-19 could potentially be used to advance the search for a non-laboratory source if these records could be made available by Chinese authorities.

---

[5] http://medanthrotheory.org/read/10867/zoonosis

[6] https://www.sciencedirect.com/science/article/pii/S2590053619300308

[7] Sensitivity of bats to urbanization: a review. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7094881/

[8] https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0135286

Genomic hallmarks of a naturally occurring coronavirus, acquired through field work and collection in the wild versus a manmade, genetically manipulated coronavirus are different and can be distinguishing. Certain genomic features can be discerned from analysis that can be used to distinguish a virus which is in the wild, living and evolving in an animal host, and a virus that is created in the laboratory via biotechnology methods, commercial kits used for genetic manipulations, and long standing principles developed and shared among academic and industrial scientists for vaccine development, antigen expression and industrial-scale growth conditions.

Some of these distinguishing features are contained in the Text-Table that follows.

Feature	Found in the Wild	Laboratory Derived
Location of source for recombination "pieces"	Within geo-local species in close relationship with each other	Can be between widely divergent species in space and time
Timing of recombination	Appear to cluster closely in time	No clustering in time; timing can appear to be occuring decades apart
Insertion of new furin cleavage site	Lysine insertion based on RNA polymerase stuttering, which generates AAA insertions	Well documented gain-of-function experiments use arginine residues as preferred amino acids
Codon utilization for the insertion	Uses codon bias expected for coronavirus found in the wild	Uses codon bias expected for human optimized conditions or as generated from commercial kits

Two papers, one concerning SARS 2002 and the other SARS-CoV19, illustrate the above features and will be discussed further below.

COVID-19 was not circulating in the community before the index case, as was the case for both MERS and SARS 2002.

Until a new human virus produces such distinctive features that routine medical surveillance can identify it as a new disease, the sporadic cases that occur are simply 'noise' in a background of influenza. But armed with diagnostic tools developed to deal with an epidemic and that can identify and distinguish the new virus from the background of human virus pathogens, analysis of specimens saved from before the epidemic can be used to determine the true chronology of the jump into humans.

SARS and MERS are community-acquired zoonotic diseases, from wild bats and domesticated camels, respectively, and are characterized by an extended period, six to twelve months, respectively, of sporadic human cases followed by an accelerated incidence of human cases that became recognized as an epidemic. This is because of the abundance and size of the intermediate host reservoir. A laboratory-acquired zoonotic disease, on the other hand, will not have a community reservoir and so pre-epidemic cases will not be seen.

The following Text-Table addresses this question.

Infection	Sporatic cases pre-epidemic?	Detailed Observations	Reference
Community-acquired SARS	Yes	SARS virus began causing sporadic self-limited outbreaks in late 2002, after which it seemingly developed sustained human-to-human transmission, and spread internationally, beginning in Apr 2003. Post-epidemic studies of SARS have revealed a low level circulation of the virus several months before it was first detected in humans in Guangdong Province of China, suggesting a period of time in which the virus adapted to human hosts.	Organization WHO. WHO issues global alert about cases of atypical pneumonia: cases of severe respiratory illness may spread to hospital staff. Geneva: The Organization; March 12, 2003 [Accessed 14 May 2014]. Available from: http://www.who.int/csr/sars/archive/2003_03_ 12/en/. 2003; Xu RH, He JF, Evans MR et al. Epidemiologic clues to SARS origin in China. Emerg Infect Dis 2004; 10:1030–1037.
Community-acquired MERS	Yes	The epidemiological curve of human MERS-CoV cases in KSA (Figure 1) reflects the occurrence of sporadic cases in 2012, low-level sustained transmission in 2013, and an epidemic spike between April and May 2014. Every month from April 2013 onward had MERS cases	Report on MERS
COVID-19	No	In Wuhan, COVID-19 testing of ILI samples (20 per week) in November and December 2019 and in the first two weeks of January 2020 found no positive results in the 2019 samples, 1 adult positive in the first week of January, and 3 adults positive in the second week of January; all children tested were negative for COVID-19 although a number were positive for influenza.	WHO Report on COVID-19

The absence of community cases for COVID-19, as noted by the WHO here, is consistent with a laboratory-acquired infection, in which there is no community zoonotic reservoir.[9]

Limited information about the geo-location of early cases places them close to the Hunan Market and the Wuhan Institute of Virology. One tool used by epidemiologists to identify the origin of a community infection is the tracking of the earliest cases in time and space. Here, despite a paper describing 41 hospitalized patients in Wuhan, the details of which hospitals they were admitted to and the chronology of their appearance at a hospital is not described.

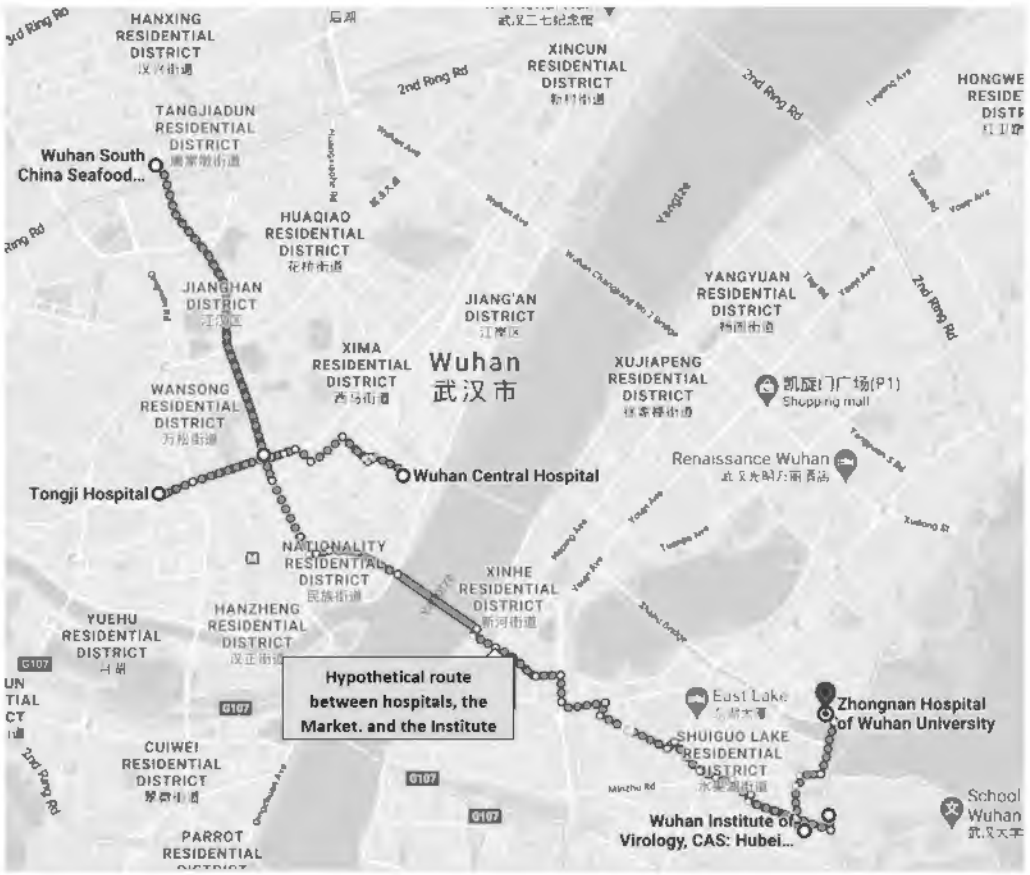
Using author affiliation as a surrogate of the hospital of admission, Beijing, where 17 authors are from, and Jin Yin-Tan Hospital, where seven authors are from, are set aside as unlikely admission locations for early cases (Jin Yin-tan Hospital was set aside because this was identified as the designated hospital where patients were "transferred to a designated hospital starting from Dec 31, 2019."

That leaves Wuhan Central Hospital, where Dr. Li Wenliang worked[10], Zhongnan Hospital[11], and Tongji Hospital. The following map shows the proximity of these three hospitals to both the Hunan Market and the Wuhan Institute of Virology. The hypothetical route shown, which includes a ferry crossing of the Yangtze River, has no evidence supporting it and is merely for illustrative purposes of the close proximity of these five locations.

[9] https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf

[10] An additional whistleblower doctor interview from Wuhan Central Hospital; https://www.theguardian.com/world/2020/mar/11/coronavirus-wuhan-doctor-ai-fen-speaks-out-against-authorities

[11] A report of 155 cases seen at Zhongnan Hospital confirms it was central to the outbreak; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7184444/



Obviously the geo-tracking data within the cell phones of the early patients could provide data to help support or refute a laboratory-derived hypothesis by mapping the location and movements of patients and their contacts.

According to a late February report from the WHO this data has already been collected: "China has a policy of meticulous case and contact identification for COVID-19. For example, in Wuhan more than 1800 teams of epidemiologists, with a minimum of 5 people/team, are tracing tens of thousands of contacts a day. Contact follow up is painstaking, with a high percentage of identified close contacts completing medical observation."[12]

A publication in the New England Journal of Medicine documented the earliest cases of COVID-19 in children. Figure S1[13] contains a map of the residences of six children and the three branches of Tongji Hospital they were admitted to. While one patient was located over 100 km from the center of Wuhan (although connected to the center with multiple daily trains trips of about 50 minutes), five surround central Wuhan. An approximate geo-center is very near the Wuhan Institute of Virology.

Evidence against a wild or domesticated animal as the source for COVID-19

[12] https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf; page 8.

[13] https://www.nejm.org/doi/suppl/10.1056/NEJMc2003717/suppl_file/nejmc2003717_appendix.pdf

The current best proposed animal sources, the bat (specimen RaTG13) and pangolin, are remotely related. Initial sequence comparisons of the human virus SARS-CoV-2 and GenBank sequence data showed sequence homology of only 91%. With a mutation rate of approximately 26 per year for SARS-CoV-2, the estimated date for divergence from the last common ancestor is *ca* 1968.

On 03 Feb 2020 a paper from the Wuhan Institute of Virology found a closer match with the findings that Bat-Co-V RaTG13, which was previously detected in *Rhinolophus affinis* from Yunnan province in 2013.

A fundamental difference between a laboratory and a non-laboratory acquired zoonotic disease, the imprint of phylogenetic diversity through pre-human spread within the source population, can be examined by the posterior diversity of human cases with no *a priori* knowledge of an intermediate host.

The MERS epidemic has been documented to have arisen from the initial jump from bats to camels, a three to five year expansion within the camel population in which mutational diversity arose by random mistakes, and then a jump into humans. This model of spread predicts that there would, at some point, be additional jumps from other camels into other patients, and a pattern of "posterior diversity," would be found in the human specimens. If the COVID-19 pandemic arose by a similar mechanism the same pattern would be seen. The following Text-Table contains such data.

Phylogenetic Feature	MERS	SARS-CoV-2
Posteriority Diversity	28/30 (93%)	0
No Posteriority Diversity	2/30 (7%)	7666
Time from first patient to first example of posterior diversity	About 60 days	None at >120 days
Depth of posterior diversity to first patient	>365 days	None

The study of MERS noted above was published in 2013 in Lancet[14] in an article entitled, "Transmission and evolution of the Middle East respiratory syndrome coronavirus in Saudi Arabia: a descriptive genomic study." Thirty specimens were used in the analysis. The features of a camel-to-human zoonotic epidemic are easily identified. Specimens taken within sixty days of the first patient, "Patient Zero," began to show a background diversity that could not be traced back through Patient Zero. The analysis of all thirty, in fact, documented that 93% were transmitted directly from the camel intermediate reservoir. And looking only at the "background" diversity permitted a calculation of the last common ancestor for the spread within the camel population of over 365 days.

A study of SARS-CoV-2[15] available May 5, 2020 and entitled, "Emergence of genomic diversity and recurrent mutations in SARS-CoV-2," looked at 7666 patient specimens from around the world for phylogenetic diversity. The authors state: "There is a robust temporal signal in the data, captured by a statistically significant correlation between sampling dates and 'root-to-tip' distances for the 7666 SARS-CoV-2 (R^2 = 0. 20, p < .001). Such positive association between sampling time and evolution is expected to arise in the presence of measurable evolution over the timeframe over which the genetic data was collected." This conclusion also argues against a MERS-like pattern of posterior diversity. In fact, the 95%

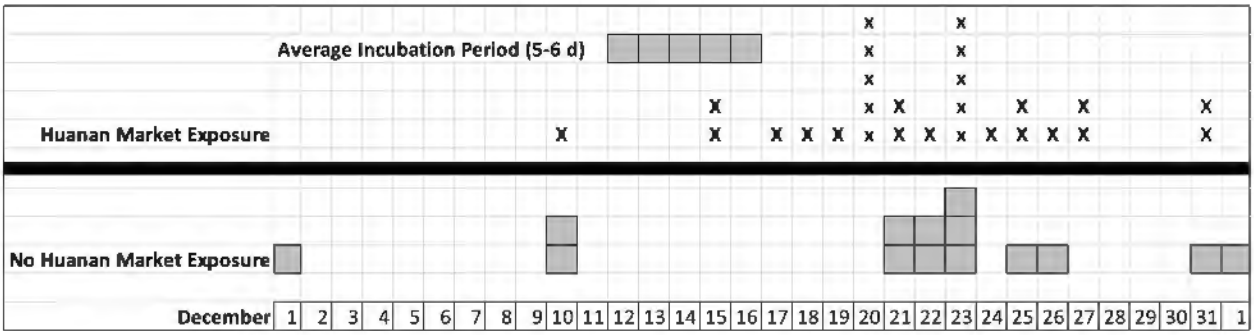
[14] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3898949/
[15] https://www.sciencedirect.com/science/article/pii/S1567134820301829

upper bound for the probability of no posterior diversity being seen in SARS-CoV-2, given the data in MERS, is 3.9×10^{-4}.

The finding of posterior diversity in MERS was seen quickly, that is, within 60 days of the first patient and in only 30 specimens. In this study of COVID-19 the cutoff date of the 7666 specimens was April 19, 2020 or approximately 140 days after the first documented case. The lack of posterior diversity in COVID-19 at a much later date than what was seen with MERS also argues against a non-laboratory source for this pandemic.

A useful avenue of future research for those working to find an animal source for COVID-19 would be new mathematical models or statistical methods that might find a "hidden" signal of posterior diversity in the current data set which shows none. And given access to the unprecedented quantity of human data for COVID-19 which can be mined via bioinformatics, efforts to find the "missing link" in the wild through search and sample should be a second priority to mining the human specimen data set.

Early cases document the Wuhan Market as an unlikely source: simple observational methods of initial cases. The first reported patient[16] was a man in his 70s with Alzheimer's disease and who lived several bus rides away and had not been to the Huanan Seafood Wholesale Market of Wuhan. His family was also unaffected and there is nothing linking him and the other cases. The calendar below shows the cases from this report and separated by those that had a connection to the market and those that did not.



Predictions which can be made from the data set of these early cases and the above simple calendar:

- The Huanan Market was not the source of SARS-CoV-2.
- Human-to-human transfer is likely.
- Cases from the market predict a five-day incubation period.[17]
- R_0 is predicted to be between 2 and 2.5.[18]
- Asymptomatic transfer seems likely.[19]

[16] https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30183-5/fulltext

[17] Using the first case on the 10th and then the next two cases on the 15th and then the jump to five cases five days later as your basis for such an estimate.

[18] This is because five days after the first case you have two and then five days later you have 5 (which means 2.5 per case). Both of these really crude estimates are quite prescient, as an April 23 New York Times article states the R0 is between 2.0 and 2.5. But on April 23rd there are 2.7 million cases worldwide to do whatever statistics you want to do!

[19] Now let's look at the cases with no Huanan Market connection. First, since the first case in the market was 10 days after the first case outside the market it didn't start in the market. Second, the non-market cases each are

- A new virus with an R_0 of 2.0-2.5, an incubation period of 5-6 days, and a not trivial incidence of asymptomatic transfer will likely become a pandemic.[20]

On January 26, 2020 in an article in *Science*[21] written by Jon Cohen, Kristian Andersen, an evolutionary biologist at the Scripps Research Institute who had analyzed sequences of 2019-nCoV to try to clarify its origin said: "The scenario of somebody being infected outside the market and then later bringing it to the market is one of the three scenarios we have considered that is still consistent with the data. It's entirely plausible given our current data and knowledge."

SARS-CoV-2 has the genomic signature of multiple recombination events, remote in time and space and unique in being cross-species, and is different from the findings with the non-laboratory derived human coronaviruses, HCoV-OC43, SARS-CoV, and MERS-CoV.

When the first genome sequence of SARS-CoV-2, Wuhan-Hu-1, was released on 10 Jan 2020 it did not cluster with any of the known bat coronavirus sequences available in public gene sequence repositories at the time or any other species. The CDC[22] reported sequence identity to SARS-CoV-2 of 85-88% for specified bat viruses, 85% for pangolin viruses, and 74-79% for civet viruses.

An apparent breakthrough in the search for a host occurred when scientists at the Wuhan Institute of Virology reported a bat *Sarbecovirus*, Bat-CoV RaTG13, that clustered with SARS-CoV-2 in almost all genomic regions, with approximately 96% overall genome sequence identity.[23] This bat virus was identified as having been collected in 2013 from Yunnan Province, about 2000 km from Wuhan, but had not previously been reported on.

Two recent papers have examined in more detail the question of the origin of SARS-CoV-2.[24] The paper entitled, "Evolutionary origins of the SARS-CoV-2 Sarbecovirus lineage responsible for the COVID-19 pandemic," provides evidence for species of origin, models the role of recombination in the viral genome, and provides estimates of the timing when it first arose.

The first observation from both papers is that the viral subgenuses responsible for the emergence of SARS-CoV-2 indicate frequent recombination. But further analysis shows SARS-CoV-2 differs in many ways from SARS-CoV. For context, data from SARS-CoV will be juxtaposed as an example of a zoonotic coronavirus from nature.

SARS-CoV. In 2017, to much media attention, Hu *et al.* published a paper[25] entitled, "Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus." The authors indicate they conducted bat samplings ten times from April 2011 to October 2015 at different

---

separated by 10 to 11 days. From these two sets of data you would predict the virus can be passed by patients who have no symptoms that are severe enough to get them to a hospital.

[20] There were 3.9 million cases on May 8, which is 168 days from the first reported case on 01 Dec 2019. Assuming an R_0 of 2.0 and an incubation period of 5.9 days one would predict 3.8 million cases.

[21] https://www.sciencemag.org/news/2020/01/wuhan-seafood-market-may-not-be-source-novel-virus-spreading-globally

[22] https://wwwnc.cdc.gov/eid/article/26/7/20-0092_article

[23] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7095418/

[24] https://www.biorxiv.org/content/10.1101/2020.03.30.015008v1.full; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7157669/

[25] https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1006698

seasons in their natural habitat at a single location (cave) in Kunming, Yunnan Province, China and curated the genomes.

From this single cave they were able to fully reconstruct the recombination history of SARS-CoV, including the highly variable regions of the hypervariable N-terminal domain (NTD), the receptor-binding domain (RBD) of the S1 gene, the ORF3 and ORF8 region, and the first discovery of bat SARSr-CoVs highly similar to human SARS-CoV in ORF3b and in the split ORF8a and 8b. With two separate recombination events, each involving three bat SARSr-CoVs and four specific recombinations, the complete human SARS-CoV was identified.

A phylogenetic analysis of these bats placed them in a single closely related clade, all of which were from this same cave in Yunnan and were distinct from another clade, all of which were collected from five other locations in Yunnan Province. The evolutionary history of human SARS-CoV was estimated[18] using "root-to-tip divergence as a function of sampling time" for non-recombining portions of the genome and was found to predict Jan 2003 as the likely timing, remarkably close to the first case in Nov 2002. The temporal signals for MERS-CoV and HCoV-OC43 were also strong and the estimate of emergence was early 2012 and *ca* 1968, respectively, and in general agreement with other methods.

SARS-CoV-2. The analysis of SARS-CoV-2 is remarkably different. Unlike the simple picture that emerged for SARS-CoV, "the SARS-CoV-2 lineage itself is not a recombinant of any viruses detected to date." Given the significant growth in the sheer number of collected and published bat virus genomes in recent years this is a remarkable statement. The authors then tried to geo-locate the multiple recombination events as best as they could. They found that they needed to propose the recombination of coronaviruses from 12 of China's 26 provinces, stretching over thousands of kilometers distance, to recreate SARS-CoV-2. A parsimonious alternative, which was not suggested, was the likely presence of representative viruses from many, if not all, of these provinces at the China Centre for Virus Culture Collection, the largest virus bank in Asia with more than 1,500 strains.[26] This Centre is housed within the Wuhan Institute of Virology.

Since there was approximately 17 years between the emergence of SARS-CoV and of SARS-CoV-2 into humans, the timing of the recombination events and SNPs that lead to SARS-CoV-2 and to SARS-CoV from their most closely related bat lineages was estimated by three different methods. The results indicate a difference of 22 $\pm$ 13 years. This suggests these two virus do not differ significantly in their proclivity to jump species.

The other paper on recombination within SARS-CoV-2 entitled, "Evolutionary Trajectory for the Emergence of Novel Coronavirus SARS-CoV-2," comes to the remarkable conclusion that "nine regions in complete genome nucleotide sequences of Wuhan-Hu-1-CoV were detected as putative recombinant regions and our recombination detection program (RDP) analysis suggested that Wuhan-Hu-1-CoV could be a recombinant of SARS (GZ02, Rf1), SARS-like (ZXC21, ZC45, W1V1) and MERS-CoVs."

Given the suggested machinations in the above papers for a process to create SARS-CoV-2 in the wild, recombining multiple bat and perhaps pangolin coronavirus genomes currently geo-located over half the land mass of China and occurring intermittently over decades of time, one has to ask themselves if there is an easier way to do this in the laboratory? The answer is yes.

---

[26] http://english.whiov.cas.cn/ne/201806/t20180604_193863.html

One of many methods for this very process was described in 2008 in a chapter[27] in *Methods in Molecular Biology* entitled, "Manipulation of the Coronavirus Genome Using Targeted RNA Recombination with Interspecies Chimeric Coronaviruses." The example given, with no sense of irony, was to create a chimeric coronavirus comprised of a mouse liver virus and a cat lung virus. To understand how prescient this chapter is, a quote from the abstract is offered:

"Key to the two-step method is the efficient selection of recombinant viruses based on host cell switching. The first step consists of the preparation—using this selection principle—of an **interspecies** chimeric coronavirus. In this virus the **ectodomain of the spike glycoprotein is replaced** by that of a coronavirus with a different species tropism. In the second step this chimeric virus is used as the recipient for recombination with synthetic donor RNA carrying the original spike gene. Recombinant viruses are then isolated on the basis of their regained natural (e.g., murine or feline) cell tropism. **Additional mutations created in the donor RNA can be co-incorporated into the recombinant virus in order to generate mutant viruses.**

(Emphasis added to illustrate how the two distinguishing features of SARS-CoV-2, the RBD mutations and the FCS insertion, are anticipated in the methodology).

To fully understand the power of this technology, the most recent common ancestor to these cat and mouse coronaviruses in nature has been estimated to have been in the year 1647 ACE[28] but with the methods contained in this chapter it can be recreated in an afternoon of laboratory work.

A key to infectivity of coronaviruses is the addition, in nature or the laboratory, of a furin cleavage site (FCS) at the S1/S2 junction of the Spike Protein.

Furin cleavage sites (FCS) have been widely understood to be important for many viral infections, including HIV, influenza, and others. It has also been widely understood before now that lineage B coronaviruses do not have FCS.

It was therefore surprising when an examination of SARS-CoV-2 Spike Protein found an insertion of a 12-nt, 4-AA sequence near the junction of the S1/S2 subunits which creates a furin site which is essential to human infectivity and transmission. As expected from previous work, no lineage B coronavirus, be it SARS-CoV, MERS, bat, pangolin, or any other, has this feature. This is the most difficult "molecular fingerprint" of SARS-CoV-2 to explain having been acquired in the wild and for that reason there are no even passingly feasible theories.

It has been known since 1994 that viral glycoproteins can be cleaved by secreted proteases, including furin.[29] Even before that, in 1992, it was known the peptide sequence R-X-K/R-R in surface glycoproteins was required for avian influenza viruses of Serotype H7 pathogenesis.[30] The first paper using furin inhibitors to define a role for an FCS in coronavirus-cell fusion was published in 2004.[31]

---

[27] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7120397/
[28] Figure 2; https://jvi.asm.org/content/81/8/4012
[29] https://www.ncbi.nlm.nih.gov/pubmed/8162439
[30] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7172898/pdf/main.pdf
[31] https://www.ncbi.nlm.nih.gov/pubmed/15141003

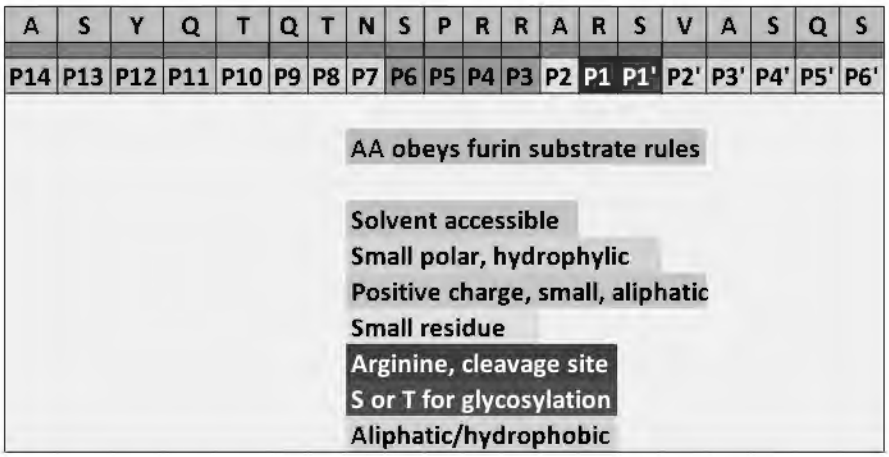
Since that time it has become common practice to insert FCS during laboratory gain-of-function experiments. The following Text-Table illustrates the scope of just a few of the experiments conducted.

URL for Paper	Title of Paper
One	Characterization of a panel of insertion mutants in human cytomegalovirus glycoprotein B.
Two	Insertion of the two cleavage sites of the respiratory syncytial virus fusion protein in Sendai virus fusion protein leads to enhanced cell-cell fusion and a decreased dependency on the HN attachment protein for activity.
Three	Recombinant Sendai viruses expressing fusion proteins with two furin cleavage sites mimic the syncytial and receptor-independent infection properties of respiratory syncytial virus.
Four	Amino acid substitutions and an insertion in the spike glycoprotein extend the host range of the murine coronavirus MHV-A59
Five	Induction of IL-8 release in lung cells via activator protein-1 by recombinant baculovirus displaying severe acute respiratory syndrome-coronavirus spike proteins: identification of two functional regions.
Six	Coronaviruses as vectors: stability of foreign gene expression.
Seven	Experimental infection of a US spike-insertion deletion porcine epidemic diarrhea virus in conventional nursing piglets and cross-protection to the original US PEDV infection.
Eight	Minimum Determinants of Transmissible Gastroenteritis Virus Enteric Tropism Are Located in the N-Terminus of Spike Protein.
Nine	Reverse genetics with a full-length infectious cDNA of the Middle East respiratory syndrome coronavirus.
Ten	Construction of a non-infectious SARS coronavirus replicon for application in drug screening and analysis of viral protein function
Eleven	A severe acute respiratory syndrome coronavirus that lacks the E gene is attenuated in vitro and in vivo.

The creation in the wild of a coronavirus FCS that is used as an example of what might have happened in SARS-CoV-2 is uninformative. In this case a strain of influenza, in which a new polybasic site appears spontaneously leads to increased infectivity and lethality,[32] was reported by Tse *et al.* 2014. The mechanism of the FCS acquisition here was an RNA polymerase dependent stuttering at a small, constrained loop in which one or more A nt were inserted, removing the strain in the loop and inserting an AAA codon which represents the basic amino acid lysine. No such method was described for the insertion of arginine.

The insert generates a canonical 20 AA furin site sequence. In 2011 Tian et al.[33] published an analysis of 126 furin cleavage sites from three species: mammals, bacteria and viruses. The analysis showed that when the furin sites are recorded as a 20-residue motif, a canonical structure emerges. It includes one core cationic region (eight amino acids, P6–P2′) and two flanking solvent accessible regions (eight amino acids, P7–P14, and four amino acids, P3′–P6′).

[32] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3911587/
[33] https://www.nature.com/articles/srep00261



This figure above shows the 20-AA of the furin motif (in green) with the P14 to P6' AA positions marked with the cleavage site being the amide bond between P1-R and the P1' residue. The motif is color coded with the requirements (in most cases, except for the positively charged AA requirements, most position requirements can be relaxed). It is interesting that Tian also indicated that many viruses have compromised residues in P3' to P6' because of having more hydrophobic residues there. SARS-CoV-2 is remarkable in having the ideal residues.

With the insertion, all 20 residues obey the rules as established by Tian. Since there are 20^4 different 4-AA peptides or 160,000 choices, it is remarkable that the 4 AA insert created a sequence that contained a small or cationic AA (8 AA/20 qualify), a cationic AA (3/20), another cationic AA (3/20), and a small AA (5/20) in that order. In fact, there are only 360 or the total or about 0.2% of all four amino acid inserts that would be expected to follow the exact rules for furin substrates. Of course, given the increase in infectivity SARS-CoV-2 has over other coronaviruses that do not have a well-designed furin cleavage site, selection pressure would drive this rare mutational event once it happened randomly.

It would also be a likely choice for a laboratory designed furin cleavage site created *de novo*.

Codon usage can distinguish insertion events in the wild from those created in the laboratory.

Not only is the insertion of an FCS peptide unique among lineage B coronaviruses, the nt sequence used for the process is more broadly unique among coronaviruses, regardless of lineage:

-CCT-**CGG-CGG**-GCA-

I will now use synonymous codon bias methods to try to inform the question of the origin of SARS-CoV-2.

Because of the redundancy of the genetic code, more than one 3-nt sequence specifies any given amino acid. For example, there are six codons that specify arginine, R. The frequencies with which such synonymous codons are used are unequal and have coevolved with the cell's translation machinery to avoid excessive use of suboptimal codons that often correspond to rare or otherwise disadvantaged tRNAs. This results in a phenomenon termed "synonymous codon bias," which varies greatly between evolutionarily distant species and possibly even between different tissues in the same species.

Decades of research has identified that all life forms, viruses, bacteria, and humans, use the codons in a signature pattern of frequency which can be used to identify a particular sequence of RNA or DNA as human or non-human; viral or non-viral.

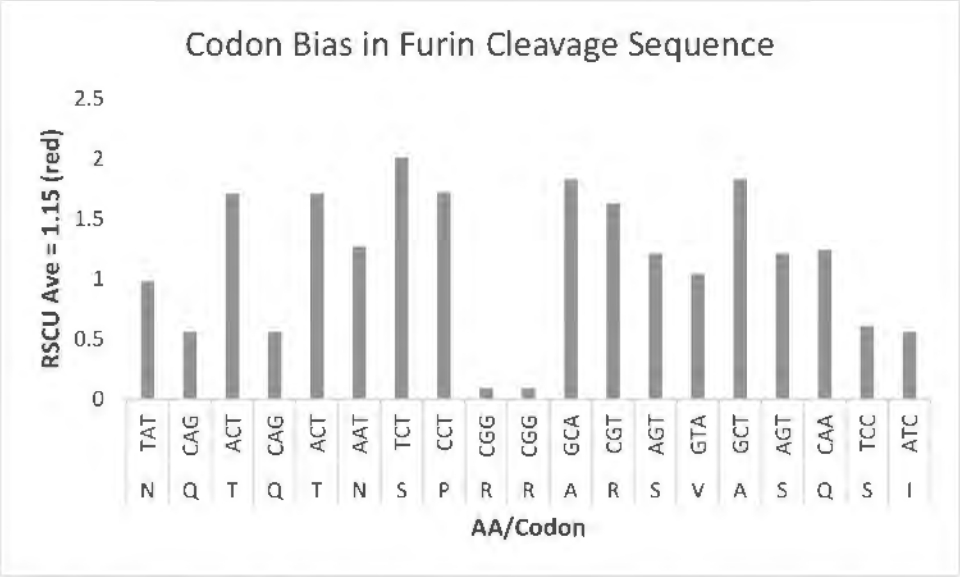
In this way, viruses in nature and scientists in the laboratory, with different goals and motivations, make distinguishing codon usage decisions which can sometimes be used as a fingerprint of their source.

The Text-Table below contains the arginine codon usage for two populations, pooled data for SARS-CoV 2003 and related viruses and 13 Sars-CoV-2 human specimens from widely dispersed locations.

Codon	SARS-CoV 2003 and ten other evolutionary related viruses in the Nidovirales	SARS-CoV-2 from 13 Geo-locations
CGG	0.09	0.09
CGA	0.44	0.37
CGC	0.72	0.37
AGG	0.9	1.07
CGU	1.77	1.63
AGA	2.08	2.48

Since these values are of a type of multiplicative scale, they were fit using a log-normal distribution, which appears appropriate (although the sample size is small). Using the log mean and standard deviation and this distribution, the probability of finding a CGG codon is about 0.024. Assuming they are independent the probability of finding a CCG-CCG codon pair is effectively 0.024^2 or 0.00058.

The following Figure shows the RSCU for the amino acids that comprise the new furin cleavage site in SARS-CoV-2. As one can see, the RSCU values are similar to each other with the exception of the RR dimer insert, which have a very low RSCU of 0.09.



The RSCU value for the CCG codon for R of 0.09 was taken from a 2004 paper of the RSCU for SARS-CoV 2003 and ten other evolutionary related viruses in the *Nidovirales* and is confirmed by 13 SARS-CoV-2 specimens obtained from diverse geographic locations. If one assumes that the RSCU observations are independent and that the probability distribution of these measurements is Gaussian (normal; a reasonable assumption), then one can calculate the probability of obtaining a result as small as 0.09. Removing the two 0.09 values, then the mean and standard deviation of the remaining values are 1.275

and 0.4992, respectively. Then the probability of a single 0.09 value is 0.0088. However, there are two 0.09 values. If we assume that these are independent findings, then the probability of both values being seen is 0.0088^2 or 7.7×10^{-5}. Using the RSCU of 0.2 from the Table above does not change the immense improbability of the usage of a CGGCGG codon pair in the wild.

Single Arginine CGG codon usage analysis suggests this will not be found in the wild.

The codon usage for SARS-CoV-2, like most coronaviruses studied, has a bias toward AT and away from GC nucleotides. The frequency of third position G use in CoV-2, for example, is 13%, 21%, 17%, and 16% for the spike protein, envelope, membrane, and nucleocapsid protein, respectively.

In that context, the scarcity of the CGG genome in SARS-CoV-2 and related coronaviruses, the relative synonymous codon usage, determined by the method of Behura and Severson,[34] was calculated and tabulated below. The color coding is blue for underutilized codons (RSCU < 1.0) and red for overutilized codons (RSCU > 1.0); light blue for RSCU values of 0.60 to 0.99 and light red for RSCU of 1.01 to 1.60. The highest RSCU usage of CGG is 1.21 in the membrane protein in the MERS virus but zero in SARS-CoV-2.

RSCU	SARS-CoV-2	Beta CoV Pangolin	SARS CoV	Bat SARS CoV	MERS CoV
Spike	0.29	0	0.19	0.08	0.25
Envelope	0	0	0	0	0
Membrane	0	0.35	0.74	0.24	1.21
Nucleocapsid	0.41	0.16	0.03	0.04	0.8

Looking at these five coronaviruses:

The largest structural protein of the coronaviruses is the spike protein, with 1273 amino acids. In SARS-CoV-2 there are 42 R residues, with only one RR dimer, the one in the insert that created SARS-CoV-2.

As a reminder none of these related coronaviruses have the 12 nucleotide insertion that forms the putative furin site in CoV-2. Interestingly, the pangolin coronavirus has no CCG residues in the spike protein. The significance of this is it makes the acquisition of this insert from pangolin by recombination impossible.

The smallest structural protein, the envelope protein, has 75 amino acids, including three R residues, but has no CGG codons in any of the related coronaviruses examined.

The SARS-CoV-2 membrane protein has 441 amino acids, 14 R residues and no CGG codons. Among related coronaviruses, this is the most unique finding of the four proteins for SARS-CoV-2 since the other four coronaviruses all utilize CCG to some extent in this protein. In the case of the MERS virus, this protein is the only occurrence in which this codon is overutilized.

The nucleocapsid protein has 418 amino acids and is responsible for packing the RNA genome. As expected for the role of R in protein-RNA interactions, it has 29 R residues and four RR dimers. None of the dimers use the CGGCGG sequence.

The nt usage of the 12-nt insert which forms the FCS cleavage site has a probability this sequence was selected for in the wild of one in 129,870.

---

[34] https://www.ncbi.nlm.nih.gov/pubmed/22889422

A blast search was performed for the 12-nt inserted sequence and adjacent extensions and only the SARS-CoV-2 sequences were identified.

Shortening the search to just the two CGG-CGG codons was only slightly more fruitful. The Text-Table below shows the frequency of the middle half of the insert, CGGCGG, across the genomes of all seven known human coronaviruses, as well as a specimen bovine coronavirus and the bat and pangolin coronaviruses with greatest homology to SARS-CoV-2. Only a single example, outside of the Spike Protein gene, has been found.

Furin PBCS sequence	Beta Coronavirus	Total Arginine Dimers Anywhere	CGGCGG in Spike Protein *	CGGCGG Anywhere in genome *	CCGCCG Anywhere in genome
SRRKRRS	Human CoV-HKU1 GenBank: KF686346.1	12	0	0	0
KRRSRRA	Bovine CoV-Quebec GenBank: AF220295.1	12	0	0	0
PRRARSV	**SARS-CoV-2 Wuhan reference sequence GenBank: NC_045512.2**	**16**	**1; nt 23,606**	0	0
PRSVRS	MERS-CoV NCBI Reference Sequence: NC_019843.3	21	0	0	0
NRRSRGA	Human CoV-OC43 London/2011 GenBank: KU131570.1	16	0	0	0
None	Human CoV-229E GeneBank: KF514433.1	15	0	0	0
None	Human CoV NL63 NCBI Reference Sequence: NC_005831.2	9	0	0	0
None	SARS-CoV 2003 ZJ0301 from China GenBank: DQ182595.1	17	0	0	0
None	Bat coronavirus RaTG13 GeneBank: MN996532.1	11	0	1; nt 9394	0
None	Pangolin PCoV_GX-P4L GenBank: MT040333.1	10	0	0	0
	Total	139	1	0	0

* - Includes both in phase codons as well as out of phase, frameshift codons.

To understand what this means for the search for the zoonotic source for SARS-CoV-2, a statistical approach was taken. Using the data from the nine viruses other than SARS-COV-2 there was a single incidence of the CGGCGG found in the bat coronavirus. Assuming 10,000 codons per genome, the frequency of CGGCGG in coronaviruses can be estimated at 2 per 45,000 codons or 4×10^{-5}. Therefore, the frequency of finding the center half of the SARS-CoV-2 insert is very small. This is consistent with the strong bias in all coronaviruses to place an A/U nt in the third codon position.

A similar analysis for the spike protein gene can be done. Since there are no instances of CGGCGG in the spike protein genome, and the gene is 3819 nucleotides long, there are 636 pairs of codons Thus, over the 9 other viruses, there are 5724 pairs of codons and no cases of the CGGCGG pair. To calculate the upper bound on the probability of such a pair from these data, one can use the Poisson "Rule of Three", which yields a value of 3/5724 or 0.00052 with 95% confidence. Now examining the SARS-COV-2 genome, there was 1 instance of the pair in question out of 636 pairs. The probability of this happening if the true rate of this occurrence for a beta coronavirus is 0.00052 is 0.044. Obviously for smaller assumed rates of this occurrence, this would result in probabilities less than 0.044.

Since the 12-nt insert has been found nowhere in the coronavirus genomic universe, examining over 300,000 sequences and using the Poisson "Rule of Three" again, the upper bound on the frequency that it exists in nature is less than one in 100,000 with 95% confidence.

This observation in conjunction with the lack of finding the 12-nt sequence in any candidate zoonotic species makes unlikely a natural source for the virus. One line of investigation to establish a wild source for this infection would be to find a coronavirus strain with the 12-nt sequence in the wild somewhere. The fact that 10 of the 12 nts are either G or C coupled with the documented bias against GC suggests this search will futile.

Laboratory codon optimization work. Codon optimization by recombinant methods (that is, to bring a gene's synonymous codon use into correspondence with the host cell's codon bias) has been widely used to improve cross-species expression of protein.

Though the opposite objective of reducing expression by intentional introduction of suboptimal synonymous codons has not been extensively investigated, isolated reports indicate that replacement of natural codons by rare codons can reduce the level of gene expression in different organisms. For example, one approach to vaccine development is to create an attenuated virus which comprises a modified viral genome containing nucleotide substitutions engineered in multiple locations in the genome, wherein the substitutions introduce synonymous de-optimized codons.

In US Patent 9,476,032[35] titled, "Attenuated viruses useful for vaccines," they state: "In one high-priority redesigned virus, most or all Arg codons are changed to CGC or **CGG** (two frequent human codons). This does not negatively affect translation." The patent contains numerous codon usages optimized for vaccine production, including the SARS-CoV virus, and in fact they use the CGG-CGG codon pair 45 times.

Beginning with a paper in 2004,[36] one motivation for codon-optimized SARS genomes is stated here: "The gene encoding the S protein of SARS-CoV contains many codons used infrequently in mammalian genes for efficiently expressed proteins. We therefore generated a codon-optimized form of the S-protein gene and compared its expression with the S-protein gene of the native viral sequence. S protein was readily detected in HEK293T cells transfected with a plasmid encoding the codon-optimized S protein."

Since that time human optimized codons have been frequently used for coronavirus research, mostly in gain-of-function experiments. In that context the "molecular fingerprint" of CGG for R is one of those common laboratory reagent gene manipulators.

Other examples:

Examples of the use of CCG codon for arginine in coronavirus research	**Reference**
SARS was genetically modified to improve ACE2 binding using "human optimized" codons, like CGG for arginine, to grow better in the laboratory. The strains were more infective.Preparation of SARS-CoV S protein pseudotyped virus. "The full-length cDNA of the SARS-CoV S gene was optimized according to human codon usage and cloned into the pCDNA3.1(+) vector (Invitrogen). The resulting "humanized" S sequence was identical with that of strain BJ01 at the amino acid level."	Wu, K. et al. Mechanisms of Host Receptor Adaptation by Severe Acute Respiratory Syndrome Coronavirus. J Biol Chem. 2012 Mar 16; 287(12): 8904–8911.

[35] http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=9476032.PN.&OS=PN/9476032&RS=PN/9476032

[36] https://www.ncbi.nlm.nih.gov/pubmed/15367630

Predictions of future evolution of a virus are a difficult, if not completely impossible, task. However, our detailed structural analysis of the host receptor adaptation mutations in SARS-CoV RBD has allowed us to predict, design, and test optimized SARS-CoV RBDs that may resemble future evolved forms of the virus. "RBD might evolve into the human-optimized form by acquiring two mutations at the 442 and 472 position." SARS-CoV-2 acquired the mutation at position 472.	Fang Li. Receptor recognition and cross-species infections of SARS coronavirus. Antiviral Res. 2013 Oct; 100(1): 246–254.
Plasmid encoding a codon-optimized form of the SARS-CoV S protein of the TOR2 i	Wenhui Li, Chengsheng Z, et al., Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2. EMBO J. 2005 Apr 20; 24(8): 1634–1643.
The gene encoding the S protein of SARS-CoV contains many codons used infrequently in mammalian genes for efficiently expressed proteins. We therefore generated a codon-optimized form of the S-protein gene and compared its expression with the S-protein gene of the native viral sequence. S protein was readily detected in HEK293T cells transfected with a plasmid encoding the codon-optimized S protein (Fig. (Fig.1).1). No S protein was detected in cells transfected with a plasmid encoding the native S-protein gene.	Moore, MJ, Dorfman, T. Retroviruses Pseudotyped with the Severe Acute Respiratory Syndrome Coronavirus Spike Protein Efficiently Infect Cells Expressing Angiotensin-Converting Enzyme 2. J Virol. 2004 Oct; 78(19): 10628–10635.
Published in 2019 by Dr. Zhengl-Li Shi, entitled "Origin and evolution of pathogenic coronaviruses," reviews genetic optimized SARS viruses using human codons	Cui, J, Fang, L. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol. 2019; 17(3): 181–192.
In 2006, Montana scoentists put a synthetic furin cleavage site into a SARS coronavirus by adding an R residue at position R667.. They write: "We show that furin cleavage at the modified R667 position generates discrete S1 and S2 subunits and potentiates membrane fusion activity." Mutations were introduced by using QuikChange mutagenesis (Stratagene)	Follis, KE, York, J, Nunberg, JH. Furin cleavage of the SARS coronavirus spike glycoprotein enhances cell–cell fusion but does not affect virion entry. Virology 350 (2006) 358–369
Identification of murine CD8 T cell epitopes in codon-optimized SARS-associated coronavirus spike protein is the title of a paper that shows that the expression of spike protein in vitro was greatly increased by expression cassette optimization.	Zhia, Y, Kobinger, GP, Jordan, H, et al. Identification of murine CD8 T cell epitopes in codon-optimized SARS-associated coronavirus spike protein

As for the human clec4C_1 and mouse clec14A, they showed very similar profiles with spike genes, especially with bat SARS-CoV, in the arginine coding groups, showing the high RSCU values over 2.50 in AGA.	Ahn,I, Jeong, B-J, Son, HS. Comparative study of synonymous codon usage variations between the nucleocapsid and spike genes of coronavirus, and C-type lectin domain genes of human and mouse. Experimental & Molecular Medicine volume 41, pages746–756, 2009.

One locally relevant paper,[37] in which arginine residues were being inserted into bovine herpesvirus-1, used primers to create RR dimers with nine separate -CGG-CGG- codon pairs as testament to their broad use in the laboratory.

Scientists from the Wuhan Institute of Virology provided the scientific community with a technical bulletin on how to make genetic inserts in coronaviruses and proposed using the very tool that would insert this CGGCGG codon.

A Technical Appendix entitled, "Detailed methods and primer sequences used in a study of genetically diverse filoviruses in Rousettus and Eonycteris spp. bats, China, 2009 and 2015, by Yang, Xinglou & Zhang, Yunzhi & Jiang, Ren-Di & Guo, Hua & Zhang, Wei & Li, Bei & Wang, Ning & Wang, Li & Rumberia, Cecilia & Zhou, Ji-Hua & Li, Shi-Yue & Daszak, Peter & Wang, Lin-Fa & Shi, Zheng-Li. (2017), from the Wuhan Institute of Virology identifies primer sequences for doing genetic experiments in coronaviruses and identifies CGG containing primers when a R amino acid is being inserted.

Mutational SNP and microindel analysis of SARS-CoV-2 specimens obtained from COVID-19 patients documents the essential nature of the inserted FCS, identifies unexpected criticality of other aspects of the genome within and downstream from the FCS, illustrates the selection pressure for G/C to A/T mutations in the third codon position of the CGGCGG insert, provides an example of a 9-nt insert with a template elsewhere in the genome sequence and examples of polymerase stutter with AAA insertions, and identifies a high frequency SNP which may be important for vaccine design.

Mutational analysis of a viral genome during an expanding epidemic can provide an "experiment of nature" that permits an assessment of the metes and bounds of a particular virus and can be used to view the evolutionary clock backwards. In this way we can test theories about how a virus arose and dismiss hypotheses that can be ruled out by actual genomic observations and sequence data.

A mutational analysis similar to the one performed by Mercatelli and Giorgi was conducted. The 16,079 complete genomes deposited with GISAID, beginning with the Wuhan reference sequence which was deposited on 05 Jan 2020 and including genomes up to 20 Apr 2020, were collated, tabulated, and analyzed. The Table below summarizes the mutational analysis of this GISAID data.

[37] From the Wuhan Institute of Virology; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7125963/

SNPs/Insertions/ Deletions	Event	Number of events	1 nt	Frameshift	nt = 3x	x = 1	x = 2	x = 3	x = 4
SNPs	SARS-CoV-2 - 12/19 to 04/20 evolution	37,141	37,141						
Deletions	SARS-CoV-2 - 12/19 to 04/20 evolution	542		135	407				
Insertions	SARS-CoV-2 - 12/19 to 04/20 evolution	35		35	24	22	0	1	0
Insertions	Variant Y267 - Spike Protein	1				TTA/F/1.53			
Insertions	F37 - Transmembrane Protein	21				TTT/F/0.91			
Insertions	SHQ248 - Spike Protein	1						AGA/R/2.86	
Insertions	Appeared in SARS-CoV-2 for first time	1				CCT/P/2.00			GCA/A/1.37

SNP event rate. The rate of spontaneous mutations in SARS-CoV-2 was calculated assuming the time from sample collection to deposit in GISAID is 10 days, the daily worldwide caseload data permits an estimate of the median date of collection as 31 Mar 2020 or 86 days.

A total of 67,364 events in 10,014 genomes of 29,903 nt size is a rate of 9.5×10^{-4} sub per site per year. This compares well to the value of 8×10^{-4} sub per site per year that is make by NextStrain consortium.

The rate of codon-sized deletions was calculated to be 1.5×10^{-5} deletions per site per year and the rate of codon-sized insertions was 1×10^{-8} insertions per site per year. Therefore, the probability of the insertion being random but also creating a furin cleavage site among all 29,900 sites is less than 10^{-8} per year. Given estimates of the size of bat populations worldwide this is highly unlikely. This does not speak to the speed a well-placed random mutation that confers strong selection can spread through a population but only the frequency of that first event.

The rate of codon sized deletions is about 4.5% of the predicted rate for the equivalent three single nt mutation rate.

Most common functional SNP. As noted by both NextStrain as well as Mercatelli and Giorgi, the most common functional SNP (at about 17%) is A23403G located in the Spike Protein gene and responsible for a D614G AA change. This mutation has moved steadily through geographic regions, starting in Oceania, then moving to Europe, and then to North and South America and Africa. Mushala was the first to describe this mutation and a plausible structure-function impact on infectivity. The mutation Spike D614G is of urgent concern; it began spreading in Europe in early February, and when introduced to new regions it rapidly becomes the dominant form.

Kober et al performed an excellent analysis of the impact of D614G on infectivity, immunity, antibody enhanced infection, and clinical course. While it has not yet been shown to impact the clinical course of disease, the numbers analyzed were small. Even with a small study sample, Kober et al. showed it was related to faster viral load during initial nasal swab testing.

The timeline below shows how fast this mutation has penetrated the human population. It first appeared within 3-6 generations of human passage (two to three weeks). Given that it has been determined that SARS-Cov-2 has about one mutation per patient and this mutation was selected for so quickly in the mammalian host it is clear it was selected for only in a non-laboratory host setting. The importance of this is it weakens or rules out transfer from a wild animal (where it would have appeared early and, as a consequence, have been present in the first human case) to the first human.

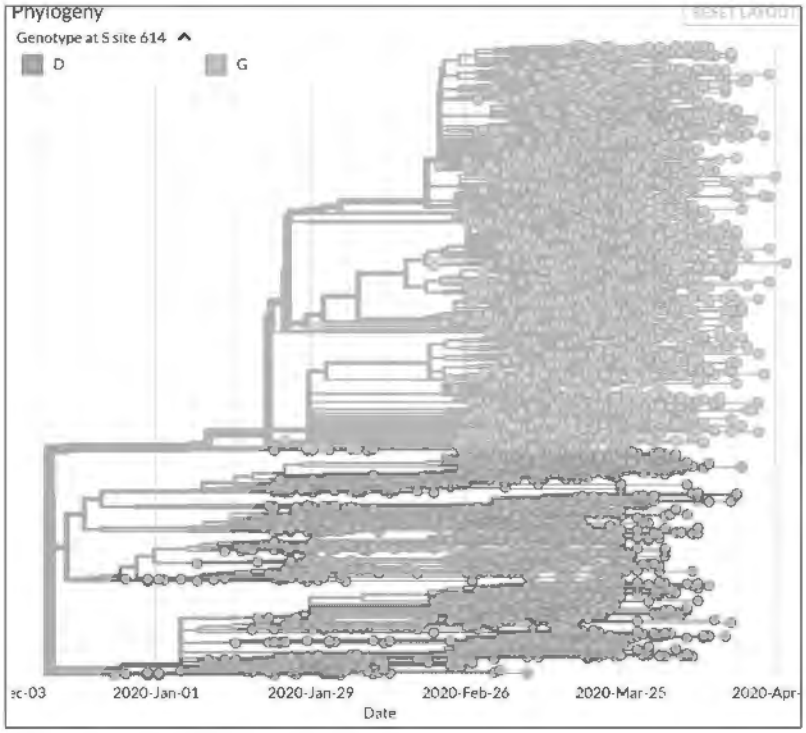


Figure above from the NextStrain website.

The finding of the SNP D614G in the Spike Protein, its appearance within two weeks of the first patient, its presence in 17% of all cases sequenced, its virtual "gene drive" like dominance outside of Asia after it formed, makes it extremely unlikely this virus could have circulated in the wild and not selected for this before human infection. The fact that this mutation provides a >20-fold increase in monkey cell growth when reintroduced there, also suggests it could not have been passaged in laboratory animals without also appearing quickly. Since growth in a tissue culture laboratory setting does not provide the same selection pressure as in a laboratory animal, in the wild, or during a human pandemic, the most likely scenario is that a laboratory scientist was growing the virus in a tissue culture cell line, probably VERO cells, and became self-infected and as such is technically "Patient Zero."

Deletions. A 30-nt deletion starting at 23,597 was documented after passage in VERO cells [EPI_ISL_417443]. The nt deletion sequence was AATTCTCCTCGGCGGGCACGTAGTGTAGCT and the corresponding AA sequence is N679SPRRARSVA. This removes the entire insertion sequence, including the FCS. Plaque purification of SARS-CoV-2 in Vero-E6 cells led to this 30-nt deletion variant. Examination of the original clinical specimen did not contain this variant. Infection of hamsters with this variant did not cause body weight loss or more severe lung pathology seen with wild type virus infection. The attenuated phenotype of this deletion documents the import of the FCS in the severity of COVID-19.

This deletion was a laboratory artifact and did not occur in a patient. Since this has never been seen in a patient it is another data point that the selection conditions in the test tube and in patients is vastly different.

Inserts. Twenty-two single codon insertions were observed which had a sequence commonality of being either TTT or TTA. This is similar to poly-A insertions seen during RNA editing mechanisms in influenza which has led to increased virulence by the acquisition of an FCS in the form of AAA coding for K. While

this has been cited by others as evidence that an FCS could have occurred for SARS-Cov-2 and been acquired in the wild, the mechanism leads exclusively to the basic AA K insertion and not the R that is seen in SARS-CoV-2.

A single insert of a three codon segment, G9410, sequence CCC-ACC-AGA and tripeptide, PTR, has been seen. As noted in the Table above, where CCC/P/0.28 means codon/AA/RSCU and red and blue designate low and high RSCU, respectively, this insert was comprised of two low frequency codons. A potential template located at G9410, GCTGGTCCA, is a reasonable candidate for an RNA polymerase switching mechanism. This position is in the ORF1ab gene, the most common gene for SNPs and the only gene in which insertions have been tolerated.

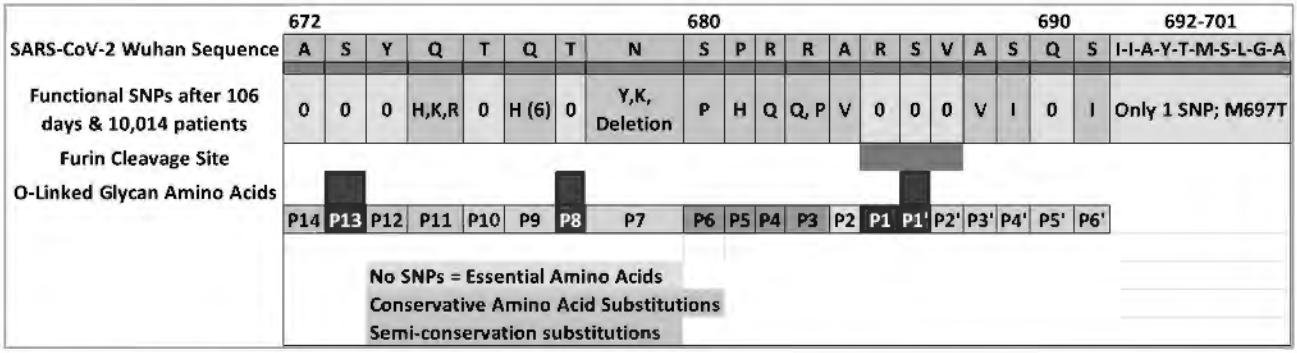
FCS mutations. The Figure below shows the 20 AA FCS and the location of mutations in the GISATD data set. Beginning with the AA without SNPs, the furin cleavage site, RS, and the two neighboring AA residues have no SNPs. The AAs on either side of the FCS center have only conservative SNP substitutions. This is strong evidence the FCS is critical to human infection.

The three AA to which sugars are post-translationally added, S673, T678, and S686, are also without functional SNPs showing the importance of these residues. T678 was not originally identified as a glycation site but the absence of SNPs suggests it has may be glycosylated, an observation important for vaccine development.

The largest sequence without a SNP presence is in the beginning of the S2 subunit, residues 692-701. With the exception of the conservative M to T substitution, this is the largest part of this region without SNPs and speaks to its import in human infection.

The Q675 to H functional SNP was seen six times, a >10-fold greater sub rate than the genome average at large. The reason for this is highly expect: all six changes are a G to T substitution, confirming the codon position 3 G aversion of SARS-CoV-2.

While this is a small data set it shows the strong bias for SARS-CoV-2 to eject a G residue in codon position 3 even at the expense of potentially reducing the efficiency of FCS cleavage. This again argues against the CCGCCG codon sequence surviving passage in the wild as a zoonotic-to-human transmission. As will be seen later, CGG is commonly used when *in vitro* growth is important as would be for a laboratory virus growing in VERO cells.



Kinetics of purification of XXG codons: resolution of the forward and reverse rate constants from SNP data.

Using GISAID data, there are six SNPs in the RR dimer codons, one a G to T change, four a G to A change, and one G to C. This is very consistent with the previous and known bias within all coronavirus, human, bat, camel, and pangolin. All of these occurred within 86 days of the first infection in a total of 10,014 genomes. This gives an imputed event rate of about 13/year, which is substantially above the mean rate of 2.7/site/year.

EPILOGUE

"Rapid reconstruction of SARS-CoV-2 using a synthetic genomics platform." An undercurrent of this analysis is the immense power for genetic manipulation that has been developed by academic and industrial scientists over the last half-century. This came to the forefront as I explained to a well-educated, non-scientist friend[38] an amazing paper.[39]

This is the *tour de force* achievement of a completely synthetic DNA gene of SARS-CoV-2 being expressed in *Saccharomyces cerevisiae,* the common Baker's yeast, and the rescue of virus following transfection into VERO cells. The 26 scientists remark that this construct was achieved within a week of receipt of the DNA fragments and then opine on ways to make it even faster.

My excitement was quickly tempered by my friend's questions and remarks.

Is this ethical he asked? Scanning the back of the paper I found this, "Ethical statement: The authors are aware that this work contains aspects of Dual Use Research of Concern (DURC). The benefits were carefully balanced against the risks and the benefits outweigh the risks."

He asked what were the benefits and risks considered but there was no more information.

He asked, "did this paper get much attention in the scientific community?" The metrics indicate the abstract has been viewed about 30,000-times. He wonders if all those people are scientists.

Being a bit of an historian he then said, "The US ended the Pacific theatre of World War II by dropping two atomic bombs on Japan. These bombs killed about 226,000 people, mostly civilians. These bombs came out of the Manhattan Project, an endeavor begun in 1939 to create the atomic bomb. It would eventually take about 130,000 scientists and others almost four years and $23 billion USD (2018 dollars) to make those two bombs.

"You're telling me 26 scientists in a lab, with a limited budget, can create SARS-CoV-2 from a bunch of chemicals, take a week to do it, and then propose ways to make it faster. The virus that has killed over 289,000 people, sickened 4.3 million, and it isn't over yet. And they do it in that little package of baker's yeast I use to make homemade bread for my family on weekends."

He got angry and said, "so the next time I go to the bakery should I ask for a little coronavirus with my sourdough bread?"

While my first instinct is to correct the misunderstandings he has about the paper, I realize he is really asking for a bigger conversation about how we got here, can we unring a bell, and where do we go from here.

---

[38] Anonymous.

[39] https://www.biorxiv.org/content/10.1101/2020.02.21.959817v1.full

U.S. DEPARTMENT OF STATE
Office of the Spokesperson

For Immediate Release

FACT SHEET

March 31, 2020

The U.S. Role in International Organizations' Response to COVID-19

"Whenever you see high quality, effective COVID-19 aid being delivered around the world by UN humanitarian and relief agencies, what you are seeing is the generosity of the American people and those who share our humanitarian values. We are by far the largest contributors to organizations like the UN Children's Fund and the World Food Program because we believe in effective multilateralism that is focused on helping those in need, not scoring political points. This is what true global leadership looks like."

-- *Secretary Michael R. Pompeo, March 27, 2020*

The United States is by far the most generous and reliable contributor to crisis response and humanitarian action through the United Nations and dozens of international organizations. U.S. assistance, monetary and in-kind contributions, expertise and technology, are indispensable to the effort to combat COVID-19. Examples include:

U.S. Support to the World Health Organization (WHO)

- The United States has been the largest supporter of the World Health Organization since its creation in 1948. **U.S. contributions to WHO in 2019 exceeded $400 million**, almost double the 2nd largest member state contribution. China, in contrast, contributed $44 million.
- WHO is coordinating the global response to the COVID-19 pandemic, and is on the ground in 149 countries around the world. This broad-based effort would not be possible without U.S. support.
- The United States and other significant partners, such as Great Britain, Germany, Japan, and the Gates Foundation, have invested for decades in WHO's emergency preparedness, response capacity, and expertise.

U.S. Support to the United Nations Children's Fund (UNICEF)

- UNICEF was one of the first organizations to provide aid to the Chinese people during the COVID-19 pandemic. On January 29, for instance, UNICEF delivered 6 metric tons of respiratory masks and protective suits for health workers to Shanghai, for distribution in Wuhan.
- This effort would not have been possible without U.S. support. **In 2019, the U.S. contributed more than $700 million to UNICEF**, compared to China's $16 million for UNICEF programs.
- UNICEF is engaged in emergency actions in dozens of countries across the globe to provide critical medical supplies, expand water, sanitation and hygiene services, and educate about means of preventing the spread of the virus.

- The United States continues to work vigorously with UNICEF to promote the health and well-being of children around the world.

U.S. Support to the United Nations Refugee Agency (UNHCR)

- Refugee populations are uniquely vulnerable to the COVID-19 pandemic, and U.S. support to UNHCR is unmatched.
- In 2019, the United States contributed nearly **$1.7 billion to UNHCR,** making it clear that we care about human suffering no matter where it occurs. China contributed $1.9 million.

U.S. Support to the World Food Program (WFP)

- The World Food Program, through its Humanitarian Response Department, has sent more than 85 shipments to 74 countries to assist in COVID-19 responses, including 1.4 million units of personal protection equipment such as health kits.
- In 2019, the United States provided 42% of WFP's $8 billion in resources, making the U.S. the largest contributor, and four times the next largest member state donation. The PRC provided just $30 million.
- Even in the midst of the pandemic, WFP maintains its goal to reach 86 million food insecure people this year – thanks to American funding and food grown by American farmers.

Stay connected with the Office of Press Relations:

Global Public Affairs
U.S. DEPARTMENT of STATE

nature medicine

A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence

Vineet D Menachery[1], Boyd L Yount Jr[1], Kari Debbink[1,2], Sudhakar Agnihothram[3], Lisa E Gralinski[1], Jessica A Plante[1], Rachel L Graham[1], Trevor Scobey[1], Xing-Yi Ge[4], Eric F Donaldson[1], Scott H Randell[5,6], Antonio Lanzavecchia[7], Wayne A Marasco[8,9], Zhengli-Li Shi[4] & Ralph S Baric[1,2]

The emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome (MERS)-CoV underscores the threat of cross-species transmission events leading to outbreaks in humans. Here we examine the disease potential of a SARS-like virus, SHC014-CoV, which is currently circulating in Chinese horseshoe bat populations[1]. Using the SARS-CoV reverse genetics system[2], we generated and characterized a chimeric virus expressing the spike of bat coronavirus SHC014 in a mouse-adapted SARS-CoV backbone. The results indicate that group 2b viruses encoding the SHC014 spike in a wild-type backbone can efficiently use multiple orthologs of the SARS receptor human angiotensin converting enzyme II (ACE2), replicate efficiently in primary human airway cells and achieve *in vitro* titers equivalent to epidemic strains of SARS-CoV. Additionally, *in vivo* experiments demonstrate replication of the chimeric virus in mouse lung with notable pathogenesis. Evaluation of available SARS-based immune-therapeutic and prophylactic modalities revealed poor efficacy; both monoclonal antibody and vaccine approaches failed to neutralize and protect from infection with CoVs using the novel spike protein. On the basis of these findings, we synthetically re-derived an infectious full-length SHC014 recombinant virus and demonstrate robust viral replication both *in vitro* and *in vivo*. Our work suggests a potential risk of SARS-CoV re-emergence from viruses currently circulating in bat populations.

The emergence of SARS-CoV heralded a new era in the cross-species transmission of severe respiratory illness with globalization leading to rapid spread around the world and massive economic impact[3,4]. Since then, several strains—including influenza A strains H5N1, H1N1 and H7N9 and MERS-CoV—have emerged from animal populations, causing considerable disease, mortality and economic hardship for the afflicted regions[5]. Although public health measures were able to stop the SARS-CoV outbreak[4], recent metagenomics studies have identified sequences of closely related SARS-like viruses circulating in Chinese bat populations that may pose a future threat[1,6]. However, sequence data alone provides minimal insights to identify and prepare for future prepandemic viruses. Therefore, to examine the emergence potential (that is, the potential to infect humaus) of circulating bat CoVs, we built a chimeric virus encoding a novel, zoonotic CoV spike protein—from the RsSHC014-CoV sequence that was isolated from Chinese horseshoe hats[1]—in the context of the SARS-CoV mouse-adapted backbone. The hybrid virus allowed us to evaluate the ability of the novel spike protein to cause disease independently of other necessary adaptive mutations in its natural backbone. Using this approach, we characterized CoV infection mediated by the SHC014 spike protein in primary human airway cells and *in vivo*, and tested the efficacy of available immune therapeutics against SHC014-CoV. Together, the strategy translates metagenomics data to help predict and prepare for future emergent viruses.

The sequences of SHC014 and the related RsWIV1-CoV show that these CoVs are the closest relatives to the epidemic SARS-CoV strains (**Fig. 1a,b**); however, there are important differences in the 14 residues that bind human ACE2, the receptor for SARS-CoV, including the five that are critical fur host range: Y442, L472, N479, T487 and Y491 (ref. 7). In WIV1, three of these residues vary from the epidemic SARS-CoV Urbani strain, but they were not expected to alter binding to ACE2 (**Supplementary Fig. 1a,b** and **Supplementary Table 1**). This fact is confirmed by both pseudotyping experiments that measured the ability of lentiviruses encoding WIV1 spike proteins to enter cells expressing human ACE2 (**Supplementary Fig. 1**) and by *in vitro* replication assays of WIV1-CoV (ref. 1). In contrast, 7 of 14 ACE2-interaction residues in SHC014 are different from those in SARS-CoV, including all five residues critical for host range (**Supplementary Fig. 1c** and **Supplementary Table 1**). These changes, coupled with

[1]Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA. [2]Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA. [3]National Center for Toxicological Research, Food and Drug Administration, Jefferson, Arkansas, USA. [4]Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China. [5]Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA. [6]Cystic Fibrosis Center, Marsico Lung Institute, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA. [7]Institute for Research in Biomedicine, Bellinzona Institute of Microbiology, Zurich, Switzerland. [8]Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA. [9]Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA. Correspondence should be addressed to R.S.B. (rbaric@email.unc.edu) or V.D.M. (vineet@email.unc.edu).

Received 12 June; accepted 8 October; published online 9 November 2015; corrected online 20 November 2015 (details online); doi:10.1038/nm.3985

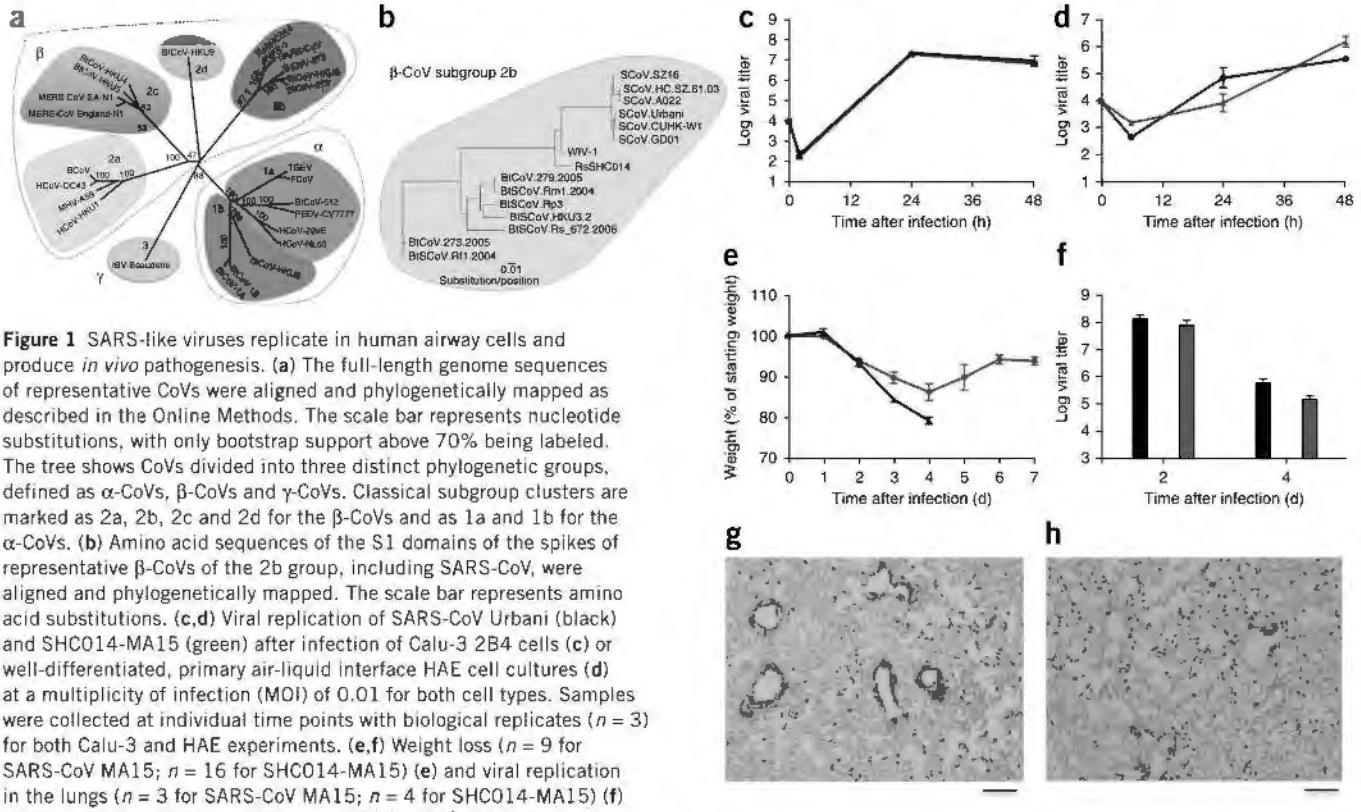


Figure 1 SARS-like viruses replicate in human airway cells and produce *in vivo* pathogenesis. (**a**) The full-length genome sequences of representative CoVs were aligned and phylogenetically mapped as described in the Online Methods. The scale bar represents nucleotide substitutions, with only bootstrap support above 70% being labeled. The tree shows CoVs divided into three distinct phylogenetic groups, defined as α-CoVs, β-CoVs and γ-CoVs. Classical subgroup clusters are marked as 2a, 2b, 2c and 2d for the β-CoVs and as 1a and 1b for the α-CoVs. (**b**) Amino acid sequences of the S1 domains of the spikes of representative β-CoVs of the 2b group, including SARS-CoV, were aligned and phylogenetically mapped. The scale bar represents amino acid substitutions. (**c**,**d**) Viral replication of SARS-CoV Urbani (black) and SHC014-MA15 (green) after infection of Calu-3 2B4 cells (**c**) or well-differentiated, primary air-liquid interface HAE cell cultures (**d**) at a multiplicity of infection (MOI) of 0.01 for both cell types. Samples were collected at individual time points with biological replicates ($n = 3$) for both Calu-3 and HAE experiments. (**e**,**f**) Weight loss ($n = 9$ for SARS-CoV MA15; $n = 16$ for SHC014-MA15) (**e**) and viral replication in the lungs ($n = 3$ for SARS-CoV MA15; $n = 4$ for SHC014-MA15) (**f**) of 10-week-old BALB/c mice infected with 1×10^4 p.f.u. of mouse-adapted SARS-CoV MA15 (black) or SHC014-MA15 (green) via the intranasal (i.n.) route. (**g**,**h**) Representative images of lung sections stained for SARS-CoV N antigen from mice infected with SARS-CoV MA15 ($n = 3$ mice) (**g**) or SHC014-MA15 ($n = 4$ mice) (**h**) are shown. For each graph, the center value represents the group mean, and the error bars define the s.e.m. Scale bars, 1 mm.

the failure of pseudotyped lentiviruses expressing the SHC014 spike to enter cells (**Supplementary Fig. 1d**), suggested that the SHC014 spike is unable to bind human ACE2. However, similar changes in related SARS-CoV strains had been reported to allow ACE2 binding[7,8], suggesting that additional functional testing was required for verification. Therefore, we synthesized the SHC014 spike in the context of the replication-competent, mouse-adapted SARS-CoV backbone (we hereafter refer to the chimeric CoV as SHC014-MA15) to maximize the opportunity for pathogenesis and vaccine studies in mice (**Supplementary Fig. 2a**). Despite predictions from both structure-based modeling and pseudotyping experiments, SHC014-MA15 was viable and replicated to high titers in Vero cells (**Supplementary Fig. 2b**). Similarly to SARS, SHC014-MA15 also required a functional ACE2 molecnle for entry and could nse human, civet and bat ACE2 orthologs (**Supplementary Fig. 2c**,**d**). To test the ability of the SHC014 spike to mediate infection of the human airway, we examined the sensitivity of the human epithelial airway cell line Calu-3 2B4 (ref. 9) to infection and found robust SHC014-MA15 replication, comparable to that of SARS-CoV Urbani (**Fig. 1c**). To extend these findings, primary human airway epithelial (HAE) cultures were infected and showed robust replication of both viruses (**Fig. 1d**). Together, the data confirm the ability of viruses with the SHC014 spike to infect human airway cells and underscore the potential threat of cross-species transmission of SHC014-CoV.

To evaluate the role of the SHC014 spike in mediating infection *in vivo*, we infected 10-week-old BALB/c mice with 10^4 plaque-forming units (p.f.n.) of either SARS-MA15 or SHC014-MA15 (**Fig. 1e–h**). Animals infected with SARS-MA15 experienced rapid weight loss and lethality by 4 d post infection (d.p.i.); in contrast, SHC014-MA15 infection produced substantial weight loss (10%) but no lethality in mice (**Fig. 1e**). Examination of viral replication revealed nearly eqnivalent viral titers from the lungs of mice infected with SARS-MA15 or SHC014-MA15 (**Fig. 1f**). Whereas lungs from the SARS-MA15–infected mice showed robust staining in both the terminal bronchioles and the lung parenchyma 2 d.p.i. (**Fig. 1g**), those of SHC014-MA15–infected mice showed reduced airway antigen staining (**Fig. 1h**); in contrast, no deficit in antigen staining was observed in the parenchyma or in the overall histology scoring, suggesting differential infection of lung tissue for SHC014-MA15 (**Supplementary Table 2**). We next analyzed infection in more snsceptible, aged (12-month-old) animals. SARS-MA15–infected animals rapidly lost weight and succumbed to infection (**Supplementary Fig. 3a**,**b**). SHC014-MA15 infection induced robust and sustained weight loss, but had minimal lethality. Trends in the histology and antigen staining patterns that we observed in young mice were conserved in the older animals (**Supplementary Table 3**). We exclnded the possibility that SHC014-MA15 was mediating infection through an alternative receptor on the basis of experiments using *Ace2*$^{-/-}$ mice, which did not show weight loss or antigen staining after SHC014-MA15 infection (**Supplementary Fig. 4a**,**b** and **Snpplementary Table 2**). Together, the data indicate that viruses with the SHC014 spike are capable of inducing weight loss in mice in the context of a virulent CoV backbone.

Given the preclinical efficacy of Ebola monoclonal antibody therapies, such as ZMApp[10], we next sought to determine the efficacy of SARS-CoV monoclonal antibodies against infection with

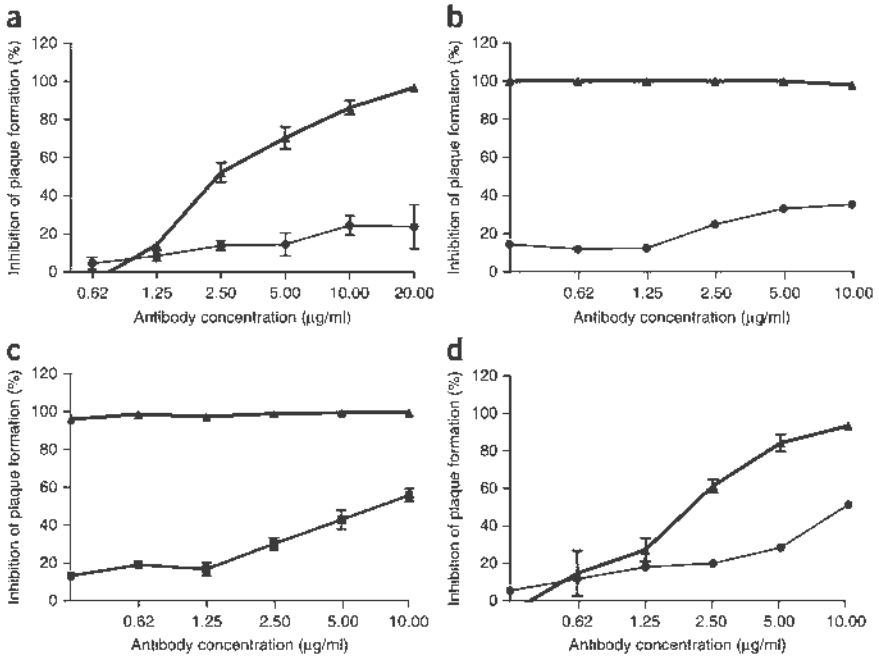


Figure 2 SARS-CoV monoclonal antibodies have marginal efficacy against SARS-like CoVs. (**a–d**) Neutralization assays evaluating efficacy (measured as reduction in the number of plaques) of a panel of monoclonal antibodies, which were all originally generated against epidemic SARS-CoV, against infection of Vero cells with SARS-CoV Urbani (black) or SHC014-MA15 (green). The antibodies tested were fm6 ($n = 3$ for Urbani; $n = 5$ for SHC014-MA15)[11,12] (**a**), 230.15 ($n = 3$ for Urbani; $n = 2$ for SHC014-MA15) (**b**), 227.15 ($n = 3$ for Urbani; $n = 5$ for SHC014-MA15) (**c**) and 109.8 ($n = 3$ for Urbani; $n = 2$ for SHC014-MA15)[13] (**d**). Each data point represents the group mean and error bars define the s.e.m. Note that the error bars in SARS-CoV Urbani–infected Vero cells in **b**,**c** are overlapped by the symbols and are not visible.

SHC014-MA15. Four broadly neutralizing human monoclonal antibodies targeting SARS-CoV spike protein had been previously reported and are probable reagents for immunotherapy[11–13]. We examined the effect of these antibodies on viral replication (expressed as percentage inhibition of viral replication) and found that whereas wild-type SARS-CoV Urbani was strongly neutralized hy all four antibodies at relatively low antibody concentrations (**Fig. 2a–d**), neutralization varied for SHC014-MA15. Fm6, an antibody generated by phage display and escape mutants[11,12], achieved only background levels of inhibition of SHC014-MA15 replication (**Fig. 2a**). Similarly, antibodies 230.15 and 227.14, which were derived from memory B cells of SARS-CoV–infected patients[13], also failed to block SIIC014-MA15 replication (**Fig. 2b,c**). For all three antibodies, differences between the SARS and SHC014 spike amino acid sequences corresponded to direct or adjacent residue changes found in SARS-CoV escape mutants (fm6 N479R; 230.15 L443V; 227.14 K390Q/E), which probahly explains the absence of the antibodies' neutralizing activity against SHC014. Finally, monoclonal antibody 109.8 was able to achieve 50% neutralization of SHC014-MA15, but only at high concentrations (10 μg/ml) (**Fig. 2d**). Together, the resnlts demonstrate that broadly neutralizing antibodies against SARS-CoV may only have marginal efficacy against emergent SARS-like CoV strains such as SHC014.

To evaluate the efficacy of existing vaccines against infection with SHC014-MA15, we vaccinated aged mice with double-inactivated whole SARS-CoV (DIV). Previons work showed that DIV could nentralize and protect young mice from challenge with a homologous virus[14]; however, the vaccine failed to protect aged animals in which augmented immune pathology was also observed, indicating the possibility of the animals being harmed because of the vaccination[15]. Here we found that DIV did not provide protection from challenge with SHC014-MA15 with regards to weight loss or viral titer (**Supplementary Fig. 5a,b**). Consistent with a previous report with other heterologous group 2b CoVs[15], serum from DIV-vaccinated, aged mice also failed to neutralize SHC014-MA15 (**Supplementary Fig. 5c**). Notably, DIV vaccination resulted in robust immune pathology (**Supplementary Table 4**) and eosinophilia (**Supplementary Fig. 5d–f**). Together, these results confirm that the DIV vaccine would not be protective against infection with SHC014 and could possibly augment disease in the aged vaccinated group.

In contrast to vaccination of mice with DIV, the use of SHC014-MA15 as a live, attenuated vaccine showed potential cross-protection against challenge with SARS-CoV, but the results have important caveats. We infected young mice with 10^4 p.f.u. of SHC014-MA15 and observed them for 28 d. We then challenged the mice with SARS-MA15 at day 29 (**Supplementary Fig. 6a**). The prior infection of the mice with the high dose of SHC014-MA15 conferred protection against challenge with a lethal dose of SARS-MA15, although there was only a minimal SARS-CoV neutralization response from the antisera elicited 28 d after SHC014-MA15 infection (**Supplementary Fig. 6b**, 1:200). In the absence of a secondary antigen boost, 28 d.p.i. represents the expected peak of antibody titers and implies that there will be diminished protection against SARS-CoV over time[16,17]. Similar results showing protection against challenge with a lethal dose of SARS-CoV were observed in aged BALB/c mice with respect to weight loss and viral replication (**Supplementary Fig. 6c,d**). However, the SHC014-MA15 infection duse of 10^4 p.f.u. induced >10% weight loss and lethality in some aged animals (**Fig. 1** and **Supplementary Fig. 3**). We found that vaccination with a lower dose of SIIC014-MA15 (100 p.f.u.), did not induce weight loss, but it also failed to protect aged animals from a SARS-MA15 lethal dose challenge (**Supplementary Fig. 6e,f**). Together, the data suggest that SHC014-MA15 challenge may confer cross-protection against SARS-CoV through conserved epitopes, but the required dose induces pathogenesis and precludes use as an attenuated vaccine.

Having established that the SHC014 spike has the ability to mediate infection of human cells and cause disease in mice, we next synthesized a full-length SHC014-CoV infections clone based on the approach used for SARS-CoV (**Fig. 3a**)[2]. Replication in Vero cells revealed no deficit for SHC014-CoV relative to that for SARS-CoV (**Fig. 3b**); however, SHC014-CoV was significantly ($P < 0.01$) attenuated in primary HAE cultures at both 24 and 48 h after infection (**Fig. 3c**). *In vivo* infection of mice demonstrated no significant weight loss but showed reduced viral replication in lungs of full-length SHC014-CoV infection, as compared to SARS-CoV Urbani (**Fig. 3d,e**). Together, the results establish the viability of full-length

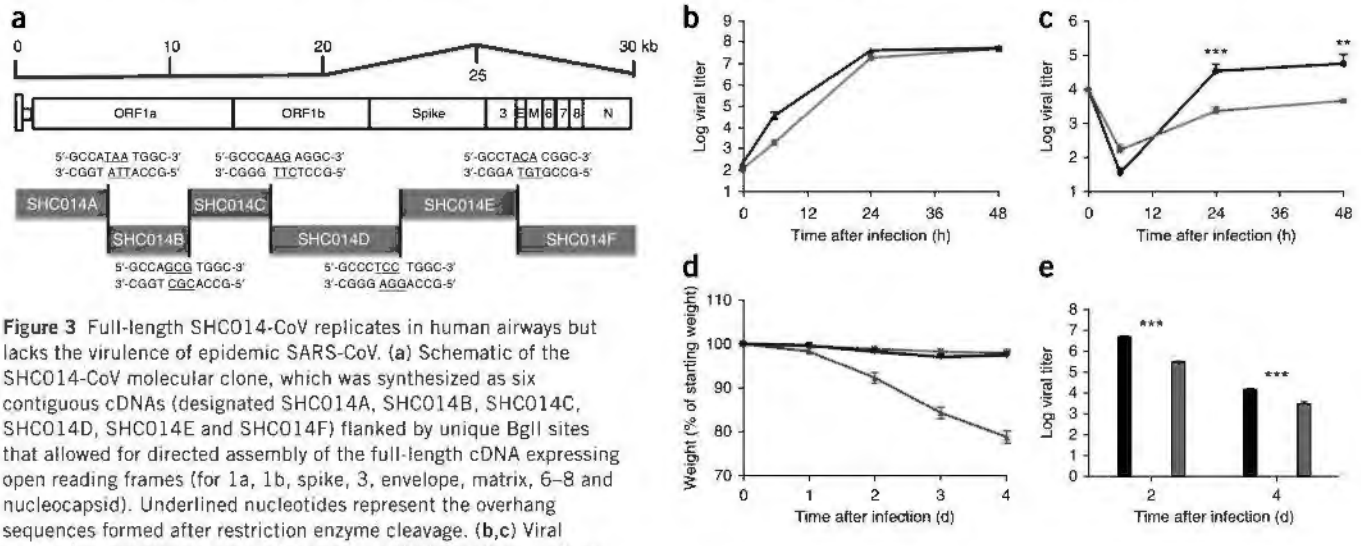


Figure 3 Full-length SHC014-CoV replicates in human airways but lacks the virulence of epidemic SARS-CoV. (**a**) Schematic of the SHC014-CoV molecular clone, which was synthesized as six contiguous cDNAs (designated SHC014A, SHC014B, SHC014C, SHC014D, SHC014E and SHC014F) flanked by unique BglI sites that allowed for directed assembly of the full-length cDNA expressing open reading frames (for 1a, 1b, spike, 3, envelope, matrix, 6–8 and nucleocapsid). Underlined nucleotides represent the overhang sequences formed after restriction enzyme cleavage. (**b**,**c**) Viral replication of SARS-CoV Urbani (black) or SHC014-CoV (green) after infection of Vero cells (**b**) or well-differentiated, primary air-liquid interface HAE cell cultures (**c**) at an MOI of 0.01. Samples were collected at individual time points with biological replicates ($n = 3$) for each group. Data represent one experiment for both Vero and HAE cells. (**d**,**e**) Weight loss ($n = 3$ for SARS-CoV MA15, $n = 7$ for SHC014-CoV; $n = 6$ for SARS-Urbani) (**d**) and viral replication in the lungs ($n = 3$ for SARS-Urbani and SHC014-CoV) (**e**) of 10-week-old BALB/c mice infected with 1×10^5 p.f.u. of SARS-CoV MA15 (gray), SHC014-CoV (green) or SARS-CoV Urbani (black) via the i.n. route. Each data point represents the group mean, and error bars define the s.e.m. $^{**}P < 0.01$ and $^{***}P < 0.001$ using two-tailed Student's *t*-test of individual time points.

SHC014-CoV, but suggest that further adaptation is required for its replication to be equivalent to that of epidemic SARS-CoV in human respiratory cells and in mice.

During the SARS-CoV epidemic, links were quickly established between palm civets and the CoV strains that were detected in humans[4]. Building on this finding, the common emergence paradigm argues that epidemic SARS-CoV originated as a bat virus, jumped to civets and incorporated changes within the receptor-binding domain (RBD) to improve binding to civet Ace2 (ref. 18). Subsequent exposure to people in live-animal markets permitted human infection with the civet strain, which, in turn, adapted to become the epidemic strain (**Fig. 4a**). However, phylogenetic analysis suggests that early human SARS strains appear more closely related to bat strains than to civet strains[18]. Therefore, a second paradigm argues that direct bat-human transmission initiated SARS-CoV emergence and that palm civets served as a secondary host and reservoir for continued infection (**Fig. 4b**)[19]. For both paradigms, spike adaptation in a secondary host is seen as a necessity, with most mutations expected to occur within the RBD, thereby facilitating improved infection. Both theories imply that pools of bat CoVs are limited and that host-range mutations are

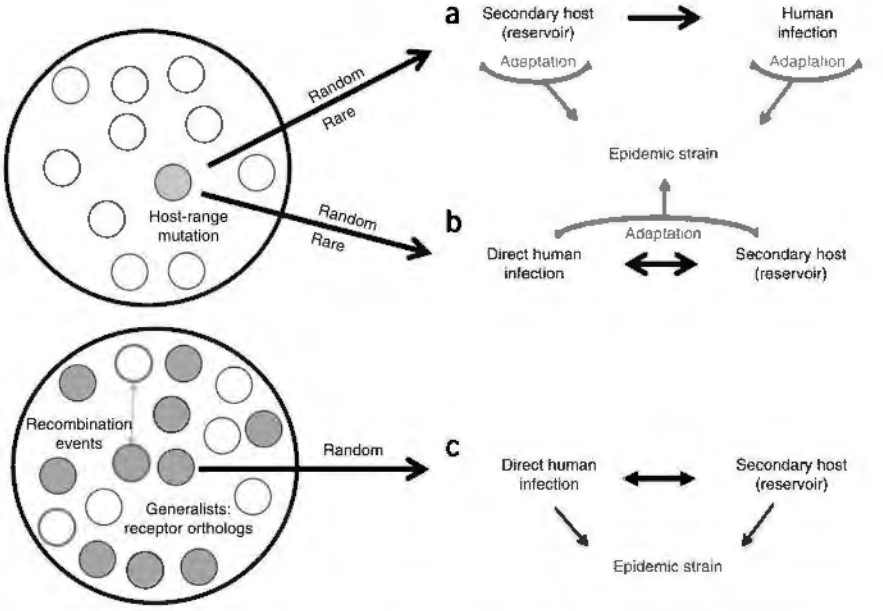


Figure 4 Emergence paradigms for coronaviruses. Coronavirus strains are maintained in quasi-species pools circulating in bat populations. (**a**,**b**) Traditional SARS-CoV emergence theories posit that host-range mutants (red circle) represent random and rare occurrences that permit infection of alternative hosts. The secondary-host paradigm (**a**) argues that a nonhuman host is infected by a bat progenitor virus and, through adaptation, facilitates transmission to humans; subsequent replication in humans leads to the epidemic viral strain. The direct paradigm (**b**) suggests that transmission occurs between bats and humans without the requirement of an intermediate host; selection then occurs in the human population with closely related viruses replicating in a secondary host, permitting continued viral persistence and adaptation in both. (**c**) The data from chimeric SARS-like viruses argue that the quasi-species pools maintain multiple viruses capable of infecting human cells without the need for mutations (red circles). Although adaptations in secondary or human hosts may be required for epidemic emergence, if SHC014 spike–containing viruses recombined with virulent CoV backbones (circles with green outlines), then epidemic disease may be the result in humans. Existing data support elements of all three paradigms.

both random and rare, reducing the likelihood of future emergence events in humans.

Although our study does not invalidate the other emergence routes, it does argue for a third paradigm in which circulating bat CoV pools maintain 'poised' spike proteins that are capable of infecting humans without mutation or adaptation (**Fig. 4c**). This hypothesis is illustrated by the ability of a chimeric virus containing the SHC014 spike in a SARS-CoV backbone to cause robust infection in both human airway cultures and in mice without RBD adaptation. Coupled with the observation of previously identified pathogenic CoV backbones[3,20], our results suggest that the starting materials required for SARS-like emergent strains are currently circulating in animal reservoirs. Notably, although full-length SHC014-CoV probably requires additional backbone adaption to mediate human disease, the documented high-frequency recombination events in CoV families underscores the possibility of future emergence and the need for further preparation.

To date, genomics screens of animal populations have primarily been used to identify novel viruses in outbreak settings[21]. The approach here extends these data sets to examine questions of viral emergence and therapeutic efficacy. We consider viruses with the SHC014 spike a potential threat owing to their ability to replicate in primary human airway cultures, the best available model for human disease. In addition, the observed pathogenesis in mice indicates a capacity for SHC014-containing viruses to cause disease in mammalian models, without RBD adaptation. Notably, differential tropism in the lung as compared to that with SARS-MA15 and attenuation of full-length SHC014-CoV in HAE cultures relative to SARS-CoV Urbani suggest that factors beyond ACE2 binding—including spike processivity, receptor bio-availability or antagonism of the host immune responses may contribute to emergence. However, further testing in nonhuman primates is required to translate these finding into pathogenic potential in humans. Importantly, the failure of available therapeutics defines a critical need for further study and for the development of treatments. With this knowledge, surveillance programs, diagnostic reagents and effective treatments can be produced that are protective against the emergence of group 2b–specific CoVs, such as SHC014, and these can be applied to other CoV branches that maintain similarly heterogeneous pools.

In addition to offering preparation against future emerging viruses, this approach must be considered in the context of the US government-mandated pause on gain-of-function (GOF) studies[22]. On the basis of previous models of emergence (**Fig. 4a,b**), the creation of chimeric virnses such as SHC014-MA15 was not expected to increase pathogenicity. Although SHC014-MA15 is attenuated relative to its parental mouse-adapted SARS-CoV, similar studies examining the pathogenicity of CoVs with the wild-type Urbani spike within the MA15 backbone showed no weight loss in mice and reduced viral replication[23]. Thus, relative to the Urbani spike–MA15 CoV, SHC014-MA15 shows a gain in pathogenesis (**Fig. 1**). On the basis of these findings, scientific review panels may deem similar studies building chimeric viruses based on circulating strains too risky to pursue, as increased pathogenicity in mammalian models cannot be excluded. Coupled with restrictions on mouse-adapted strains and the development of monoclonal antibodies using escape mutants, research into CoV emergence and therapeutic efficacy may be severely limited moving forward. Together, these data and restrictions represent a crossroads of GOF research concerns; the potential to prepare for and mitigate future outbreaks must be weighed against the risk of creating more dangerous pathogens. In developing policies moving forward, it is important to consider the value of the data generated by these studies and whether these types of chimeric virus studies warrant further investigation versus the inherent risks involved.

Overall, our approach has used metagenomics data to identify a potential threat posed by the circulating bat SARS-like CoV SHC014. Because of the ability of chimeric SHC014 viruses to replicate in human airway cultures, cause pathogenesis *in vivo* and escape current therapeutics, there is a need for both surveillance and improved therapeutics against circulating SARS-like viruses. Our approach also unlocks the use of metagenomics data to predict viral emergence and to apply this knowledge in preparing to treat future emerging virus infections.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

Research in this manuscript was supported by grants from the National Institute of Allergy & Infectious Disease and the National Institute of Aging of the US National Institutes of Health (NIH) under awards U19AI109761 (R.S.B.), U19AI107810 (R.S.B.), AI085524 (W.A.M.), F32AI102561 (V.D.M.) and K99AG049092 (V.D.M.), and by the National Natural Science Foundation of China awards 81290341 (Z.-L.S.) and 31470260 (X.-Y.G.), and by USAID-EPT-PREDICT funding from EcoHealth Alliance (Z. L.S.). Human airway epithelial cultures were supported by the National Institute of Diabetes and Digestive and Kidney Disease of the NIH under award NIH DK065988 (S.H.R.). We also thank M.T. Ferris (Dept. of Genetics, University of North Carolina) for the reviewing of statistical approaches and C.T. Tseng (Dept. of Microbiology and Immunology, University of Texas Medical Branch) for providing Calu-3 cells. Experiments with the full-length and chimeric SHC014 recombinant viruses were initiated and performed before the GOF research funding pause and have since been reviewed and approved for continued study by the NIH. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

AUTHOR CONTRIBUTIONS

V.D.M. designed, coordinated and performed experiments, completed analysis and wrote the manuscript. B.L.Y. designed the infectious clone and recovered chimeric viruses; S.A. completed neutralization assays; L.E.G. helped perform mouse experiments; T.S. and J.A.P. completed mouse experiments and plaque assays; X. Y.G. performed pseudotyping experiments; K.D. generated structural figures and predictions; E.F.D. generated phylogenetic analysis; R.L.G. completed RNA analysis; S.H.R. provided primary HAE cultures; A.L. and W.A.M. provided critical monoclonal antibody reagents; and Z.-L.S. provided SHC014 spike sequences and plasmids. R.S.B. designed experiments and wrote manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

1. Ge, X.Y. *et al.* Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **503**, 535–538 (2013).
2. Yount, B. *et al.* Reverse genetics with a full-length infectious cDNA of severe acute respiratory syndrome coronavirus. *Proc. Natl. Acad. Sci. USA* **100**, 12995–13000 (2003).
3. Becker, M.M. *et al.* Synthetic recombinant bat SARS-like coronavirus is infectious in cultured cells and in mice. *Proc. Natl. Acad. Sci. USA* **105**, 19944–19949 (2008).
4. Peiris, J.S., Guan, Y. & Yuen, K.Y. Severe acute respiratory syndrome. *Nat. Med.* **10**, S88–S97 (2004).
5. Al-Tawfiq, J.A. *et al.* Surveillance for emerging respiratory viruses. *Lancet Infect. Dis.* **14**, 992–1000 (2014).
6. He, B. *et al.* Identification of diverse alphacoronaviruses and genomic characterization of a novel severe acute respiratory syndrome–like coronavirus from bats in China. *J. Virol.* **88**, 7070–7082 (2014).
7. Li, F. Receptor recognition and cross-species infections of SARS coronavirus. *Antiviral Res.* **100**, 246–254 (2013).

8. Sheahan, T. *et al.* Mechanisms of zoonotic severe acute respiratory syndrome coronavirus host range expansion in human airway epithelium. *J. Virol.* **82**, 2274–2285 (2008).
9. Yoshikawa, T. *et al.* Dynamic innate immune responses of human bronchial epithelial cells to severe acute respiratory syndrome–associated coronavirus infection. *PLoS ONE* **5**, e8729 (2010).
10. Qiu, X. *et al.* Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. *Nature* **514**, 47–53 (2014).
11. Sui, J. *et al.* Broadening of neutralization activity to directly block a dominant antibody-driven SARS-coronavirus evolution pathway. *PLoS Pathog.* **4**, e1000197 (2008).
12. Sui, J. *et al.* Effects of human anti–spike protein receptor binding domain antibodies on severe acute respiratory syndrome coronavirus neutralization escape and fitness. *J. Virol.* **88**, 13769–13780 (2014).
13. Rockx, B. *et al.* Escape from human monoclonal antibody neutralization affects *in vitro* and *in vivo* fitness of severe acute respiratory syndrome coronavirus. *J. Infect. Dis.* **201**, 946–955 (2010).
14. Spruth, M. *et al.* A double-inactivated whole-virus candidate SARS coronavirus vaccine stimulates neutralizing and protective antibody responses. *Vaccine* **24**, 652–661 (2006).
15. Bolles, M. *et al.* A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge. *J. Virol.* **85**, 12201–12215 (2011).
16. Siegrist, C.-A. in *Vaccines* 6th edn. (eds. Plotkin, S.A., Orenstein, W.A. & Offit, P.A.) 14–32 (W.B. Saunders, 2013).
17. Deming, D. *et al.* Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants. *PLoS Med.* **3**, e525 (2006).
18. Graham, R.L., Donaldson, E.F. & Baric, R.S. A decade after SARS: strategies for controlling emerging coronaviruses. *Nat. Rev. Microbiol.* **11**, 836–848 (2013).
19. Graham, R.L. & Baric, R.S. Recombination, reservoirs and the modular spike: mechanisms of coronavirus cross-species transmission. *J. Virol.* **84**, 3134–3146 (2010).
20. Agnihothram, S. *et al.* A mouse model for betacoronavirus subgroup 2c using a bat coronavirus strain HKU5 variant. *MBio* **5**, e00047-14 (2014).
21. Relman, D.A. Metagenomics, infectious disease diagnostics and outbreak investigations: sequence first, ask questions later? *J. Am. Med. Assoc.* **309**, 1531–1532 (2013).
22. Kaiser, J. Moratorium on risky virology studies leaves work at 14 institutions in limbo. *ScienceInsider* http://news.sciencemag.org/biology/2014/10/moratorium-risky-virology-studies-leaves-work-14-institutions-limbo (2014).
23. Frieman, M. *et al.* Molecular determinants of severe acute respiratory syndrome coronavirus pathogenesis and virulence in young and aged mouse models of human disease. *J. Virol.* **86**, 884–897 (2012).

ONLINE METHODS

Viruses, cells, *in vitro* infection and plaque assays. Wild-type SARS-CoV (Urbani), mouse-adapted SARS-CoV (MA15) and chimeric SARS-like CoVs were cultured on Vero E6 cells (obtained from United States Army Medical Research Institute of Infectious Diseases), grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco, CA) and 5% fetal clone serum (FCS) (Hyclone, South Logan, UT) along with antibiotic/antimycotic (Gibco, Carlsbad, CA). DBT cells (Baric laboratory, source unknown) expressing *ACE2* orthologs have been previously described for both human and civet; bat *Ace2* sequence was based on that from *Rhinolophus leschenaulti*, and DBT cells expressing bat *Ace2* were established as described previously[8]. Pseudotyping experiments were similar to those using an HIV-based pseudovirus, prepared as previously described[10], and examined on HeLa cells (Wuhan Institute of Virology) that expressed *ACE2* orthologs. HeLa cells were grown in minimal essential medium (MEM) (Gibco, CA) supplemented with 10% FCS (Gibco, CA) as previously described[24]. Growth curves in Vero E6, DBT, Calu-3 2B4 and primary human airway epithelial cells were performed as previously described[8,25]. None of the working cell line stocks were authenticated or tested for mycoplasma recently, although the original seed stocks used to create the working stocks are free from contamination. Human lungs for HAE cultures were procured under University of North Carolina at Chapel Hill Institutional Review Board–approved protocols. HAE cultures represent highly differentiated human airway epithelium containing ciliated and non-ciliated epithelial cells as well as goblet cells. The cultures are also grown on an air-liquid interface for several weeks before use, as previously described[26]. Briefly, cells were washed with PBS and inoculated with virus or mock-diluted in PBS for 40 min at 37 °C. After inoculation, cells were washed three times and fresh medium was added to signify time '0'. Three or more biological replicates were harvested at each described time point. No blinding was used in any sample collections nor were samples randomized. All virus cultivation was performed in a biosafety level (BSL) 3 laboratory with redundant fans in the biosafety cabinets, as described previously by our group[2]. All personnel wore powered air purifying respirators (Breathe Easy, 3M) with Tyvek suits, aprons and booties and were double-gloved.

Sequence clustering and structural modeling. The full-length genomic sequences and the amino acid sequences of the S1 domains of the spike of representative CoVs were downloaded from Genbank or Pathosystems Resource Integration Center (PATRIC), aligned with ClustalX and phylogenetically compared by using maximum likelihood estimation using 100 bootstraps or by using the PhyML (https://code.google.com/p/phyml/) package, respectively. The tree was generated using maximum likelihood with the PhyML package. The scale bar represents nucleotide substitutions. Only nodes with bootstrap support above 70% are labeled. The tree shows that CoVs are divided into three distinct phylogenetic groups defined as α CoVs, β CoVs and γ CoVs. Classical subgroup clusters are marked as 2a, 2b, 2c and 2d for β-CoVs, and 1a and 1b for the α-CoVs. Structural models were generated using Modeller (Max Planck Institute Bioinformatics Toolkit) to generate homology models for SHC014 and Rs3367 of the SARS RBD in complex with ACE2 based on crystal structure 2AJF (Protein Data Bank). Homology models were visualized and manipulated in MacPyMol (version 1.3).

Construction of SARS-like chimeric viruses. Both wild-type and chimeric viruses were derived from either SARS-CoV Urbani or the corresponding mouse-adapted (SARS-CoV MA15) infectious clone (ic) as previously described[27]. Plasmids containing spike sequences for SHC014 were extracted by restriction digest and ligated into the E and F plasmid of the MA15 infectious clone. The clone was designed and purchased from Bio Basic as six contiguous cDNAs using published sequences flanked by unique class II restriction endonuclease sites (BglI). Thereafter, plasmids containing wild-type, chimeric SARS-CoV and SHC014-CoV genome fragments were amplified, excised, ligated and purified. *In vitro* transcription reactions were then preformed to synthesize full length genomic RNA, which was transfected into Vero E6 cells as previously described[2]. The medium from transfected cells was harvested and served as seed stocks for subsequent experiments. Chimeric and full-length viruses were confirmed by sequence analysis before use in these studies. Synthetic construction of chimeric mutant and full length SHC014-CoV was approved by the University of North Carolina Institutional Biosafety Committee and the Dual Use Research of Concern committee.

Ethics statement. This study was carried out in accordance with the recommendations for the care and use of animals by the Office of Laboratory Animal Welfare (OLAW), NIH. The Institutional Animal Care and Use Committee (IACUC) of The University of North Carolina at Chapel Hill (UNC, Permit Number A-3410-01) approved the animal study protocol (IACUC #13-033) used in these studies.

Mice and *in vivo* infection. Female, 10-week-old and 12-month-old BALB/cAnNHsD mice were ordered from Harlan Laboratories. Mouse infections were done as previously described[20]. Briefly, animals were brought into a BSL3 laboratory and allowed to acclimate for 1 week before infection. For infection and live-attenuated virus vaccination, mice were anesthetized with a mixture of ketamine and xylazine and infected intranasally, when challenged, with 50 μl of phosphate-buffered saline (PBS) or diluted virus with three or four mice per time point, per infection group per dose as described in the figure legends. For individual mice, notations for infection including failure to inhale the entire dose, bubbling of inoculum from the nose, or infection through the mouth may have led to exclusion of mouse data at the discretion of the researcher; post-infection, no other pre-established exclusion or inclusion criteria are defined. No blinding was used in any animal experiments, and animals were not randomized. For vaccination, young and aged mice were vaccinated by footpad injection with a 20 μl volume of either 0.2 μg of double-inactivated SARS-CoV vaccine with alum or mock PBS; mice were then boosted with the same regimen 22 d later and challenged 21 d thereafter. For all groups, as per protocol, animals were monitored daily for clinical signs of disease (hunching, ruffled fur and reduced activity) for the duration of the experiment. Weight loss was monitored daily for the first 7 d, after which weight monitoring continued until the animals recovered to their initial starting weight or displayed weight gain continuously for 3 d. All mice that lost greater than 20% of their starting body weight were ground-fed and further monitored multiple times per day as long as they were under the 20% cutoff. Mice that lost greater than 30% of their starting body weight were immediately sacrificed as per protocol. Any mouse deemed to be moribund or unlikely to recover was also humanely sacrificed at the discretion of the researcher. Euthanasia was performed using an isoflurane overdose and death was confirmed by cervical dislocation. All mouse studies were performed at the University of North Carolina (Animal Welfare Assurance #A3410-01) using protocols approved by the UNC Institutional Animal Care and Use Committee (IACUC).

Histological analysis. The left lung was removed and submerged in 10% buffered formalin (Fisher) without inflation for 1 week. Tissues were embedded in paraffin and 5-μm sections were prepared by the UNC Lineberger Comprehensive Cancer Center histopathology core facility. To determine the extent of antigen staining, sections were stained for viral antigen using a commercially available polyclonal SARS CoV anti nucleocapsid antibody (Imgenex) and scored in a blinded manner by for staining of the airway and parenchyma as previously described[20]. Images were captured using an Olympus BX41 microscope with an Olympus DP71 camera.

Virus neutralization assays. Plaque reduction neutralization titer assays were performed with previously characterized antibodies against SARS-CoV, as previously described[11–13]. Briefly, neutralizing antibodies or serum was serially diluted twofold and incubated with 100 p.f.u. of the different infectious clone SARS-CoV strains for 1 h at 37 °C. The virus and antibodies were then added to a 6-well plate with 5×10^5 Vero E6 cells/well with multiple replicates ($n \geq 2$). After a 1-h incubation at 37 °C, cells were overlaid with 3 ml of 0.8% agarose in medium. Plates were incubated for 2 d at 37 °C, stained with neutral red for 3 h and plaques were counted. The percentage of plaque reduction was calculated as (1 (no. of plaques with antibody/no. of plaques without antibody)) × 100.

doi:10.1038/nm.3985

Corrigendum: Multiphoton imaging reveals a new leukocyte recruitment paradigm in the glomerulus

Sapna Devi, Anqi Li, Clare L V Westhorpe, Camden Y Lo, Latasha D Abeynaike, Sarah L Snelgrove, Pam Hall, Joshua D Ooi, Christopher G Sobey, A Richard Kitching & Michael J Hickey

Nat. Med. 19, 107–112 (2013); published online 16 December 2012; corrected after print 12 August 2015

In the published article, in the Online Methods section, it is stated that the dose of DHE used is 20 mg/kg, when in fact DHE was administered at 2 mg/kg. The error has been corrected in the HTML and PDF versions of the article.

Corrigendum: PAR1 signaling regulates the retention and recruitment of EPCR-expressing bone marrow hematopoietic stem cells

Shiri Gur-Cohen, Tomer Itkin, Sagarika Chakrabarty, Claudine Graf, Orit Kollet, Aya Ludin, Karin Golan, Alexander Kalinkovich, Guy Ledergor, Eitan Wong, Elisabeth Niemeyer, Ziv Porat, Ayelet Erez, Irit Sagi, Charles T Esmon, Wolfram Ruf & Tsvee Lapidot

Nat. Med. 21, 1307–1317 (2015); published online 12 October 2015; corrected after print 18 November 2015

In the version of this article initially published, the first author's name was incorrect. The error has been corrected in the HTML and PDF versions of the article.

Corrigendum: Myeloid-derived growth factor (C19orf10) mediates cardiac repair following myocardial infarction

Mortimer Korf-Klingebiel, Marc R Reboll, Stefanie Klede, Torben Brod, Andreas Pich, Felix Polten, L Christian Napp, Johann Bauersachs, Arnold Ganser, Eva Brinkmann, Ines Reimann, Tibor Kempf, Hans W Niessen, Jacques Mizrahi, Hans-Joachim Schönfeld, Antonio Iglesias, Maria Bobadilla, Yong Wang & Kai C Wollert

Nat. Med. 21, 140–149 (2015); published online 12 January 2015; corrected after print 19 November 2015

In the version of this article initially published, the article number in reference 13 is incorrectly stated as '100ra190' and should be '100ra90'. The error has been corrected in the HTML and PDF versions of the article.

Corrigendum: A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence

Vineet D Menachery, Boyd L Yount Jr, Kari Debbink, Sudhakar Agnihothram, Lisa E Gralinski, Jessica A Plante, Rachel L Graham, Trevor Scobey, Xing-Yi Ge, Eric F Donaldson, Scott H Randell, Antonio Lanzavecchia, Wayne A Marasco, Zhengli-Li Shi & Ralph S Baric

Nat. Med.; doi:10.1038/nm.3985; corrected 20 November 2015

In the version of this article initially published online, the authors omitted to acknowledge a funding source, USAID-EPT-PREDICT funding from EcoHealth Alliance, to Z.-L.S. The error has been corrected for the print, PDF and HTML versions of this article.

Corrigendum: Long-term glycemic control using polymer-encapsulated human stem cell–derived beta cells in immune-competent mice

Arturo J Vegas, Omid Veiseh, Mads Gürtler, Jeffrey R Millman, Felicia W Pagliuca, Andrew R Bader, Joshua C Doloff, Jie Li, Michael Chen, Karsten Olejnik, Hok Hei Tam, Siddharth Jhunjhunwala, Erin Langan, Stephanie Aresta-Dasilva, Srujan Gandham, James J McGarrigle, Matthew A Bochenek, Jennifer Hollister-Lock, Jose Oberholzer, Dale L Greiner, Gordon C Weir, Douglas A Melton, Robert Langer & Daniel G Anderson

Nat. Med.; doi:10.1038/nm.4030; corrected online 18 February 2016

In the version of this article initially published online, the authors omitted acknowledgment recognizing the histology core of the Harvard Stem Cell Institnte and several individuals for their assistance. The error has been corrected for the print, PDF and HTML versions of this article.

REUTERS

How the novel coronavirus has evolved

The world is now dealing with a different type of SARS-CoV-2 than the one that emerged in China almost a year ago, with mutations creating at least seven strains of the virus so far.

By **Jitesh Chowdhury, Simon Scarr** and **Jane Wardell**

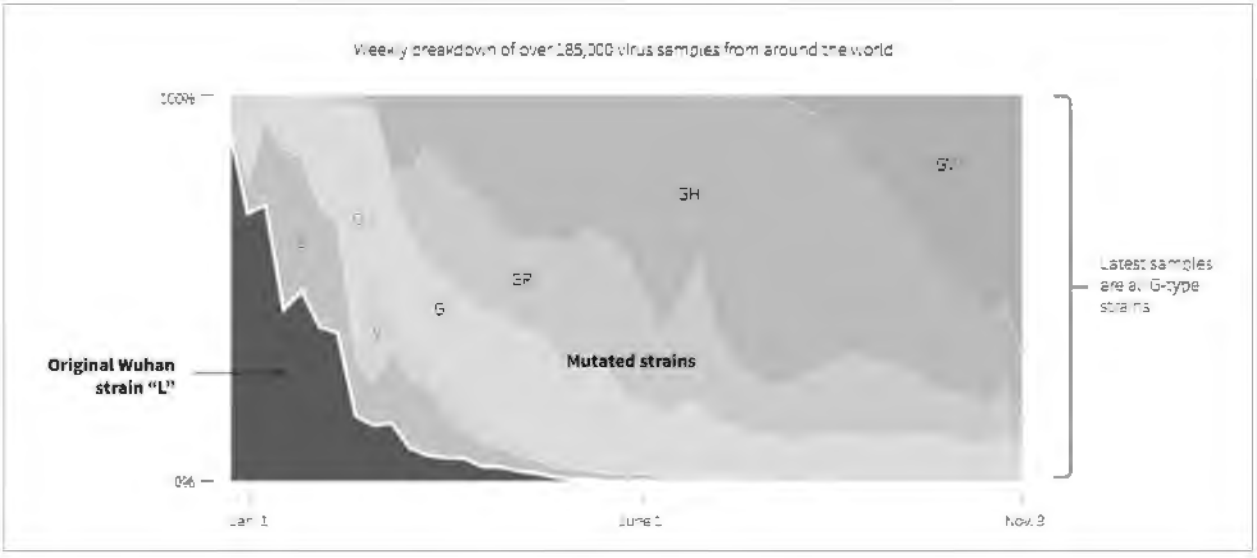
PUBLISHED DEC. 10, 2020

As the coronavirus SARS-CoV-2 swept across the world and killed more than 1.5 million in the past year, it has mutated into several major groups, or strains, as it adapted to its human hosts. Mapping and understanding those changes to the virus is crucial to developing strategies to combat the COVID-19 disease it causes.

Reuters analysed over 185,000 genome samples from the Global Initiative on Sharing All influenza Data (GISAID), the largest database of novel coronavirus genome sequences in the world, to show how the global dominance of major strains has shifted over time.

The analysis shows there are currently seven main strains of the virus. The original strain, detected in the Chinese city of Wuhan in December 2019, is the L strain. The virus then mutated into the S strain at the beginning of 2020. That was followed by V and G strains. Strain G mutated yet further into strains GR, GH and GV. Several other infrequent mutations were collectively grouped together as strain O.

Weekly breakdown of over 185,000 virus samples from around the world



"The reason for looking at the genomics is to try and find out where it came from … in terms of trying to map out what we would expect for the pandemic, that information is critical," South Australia's chief health officer, Nicola Spurrier, said following an outbreak in the state in early November. Health officials initially locked down the state because they thought the outbreak was caused by a much more contagious strain of the virus. They lifted the lockdown a day later when it turned out that a pizza restaurant worker had lied about how he caught the disease.

The graphic above shows how the original L strain is almost gone, leaving G strains dominant in the current stage of the pandemic. That's important because the G strains include one mutation that makes it easier for the spike proteins on SARS-CoV-2 to bind to receptors on human cells, potentially increasing the chances of infection and transmissibility of the virus.

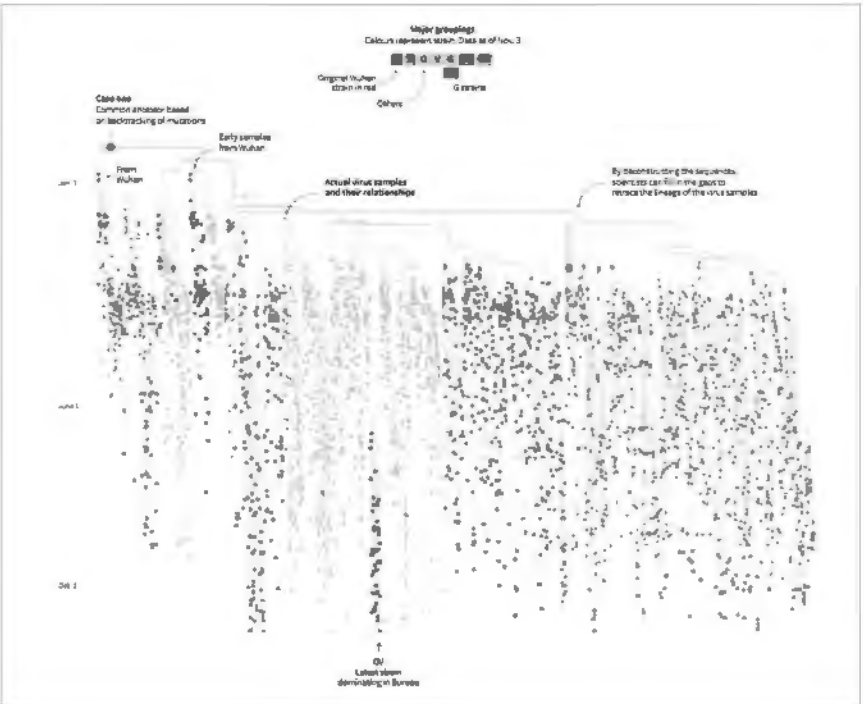
Tracking mutations

A mutation is a change in an organism's genetic material. When a virus makes millions of copies of itself and moves from host to host, not every copy is identical. These small mutations accumulate as the virus is passed on – and copied again and again.

Databases like GISAID can track these changes in individual samples, allowing scientists to connect the dots with other samples and determine when major new strains form.

The GISAID database mapped out about 3,500 of these samples from all over the world, constructing a family tree that shows how they are related. A visualisation of the data illustrates the relationships among the samples and where new strains emerged.

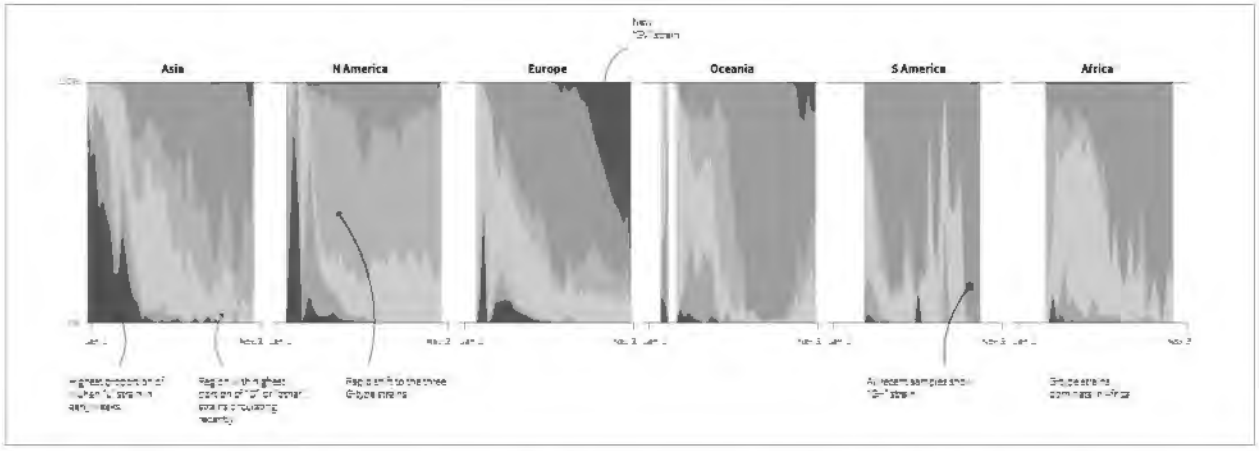
Major groupings



Shifting strains

Earlier in the pandemic, the virus made its way relatively quickly around the world, being repeatedly introduced to different locations and sparking fresh outbreaks regularly. During that time, there was a more diverse mixture of strains among the samples reported to GISAID. As countries began to close their borders, there were fewer new strains introduced. In countries where the more resilient G-type strains were present, they began to dominate.

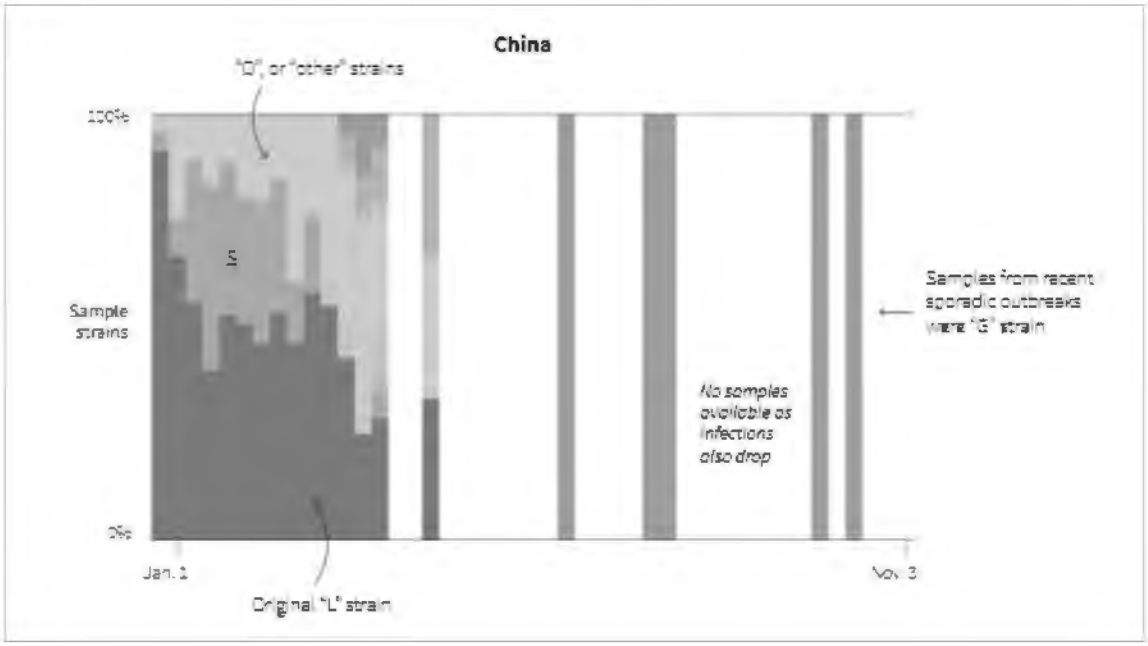
However, the timing and rate of evolution into new strains occurred at different stages for different countries and regions. Those differing patterns largely reflected how quickly the virus was able to spread in any given region and whether an outbreak was sparked by an "imported" case of the virus.



In Asia, the original L strain persisted for longer as several countries, including China, were quick to shut borders and curtail movement. In contrast, North America and Europe did not restrict movement as much, at least initially, which allowed the G strains to spread - and mutate - at a faster pace.

"A lot of it comes down to place and getting a foothold in a new population," said Catherine Bennett, epidemiology chair in the Faculty of Health at Melbourne's Deakin University. "This virus moves in superspreader events, which means the virus doesn't have to be particularly contagious. We will see different patterns because of cluster transmission."

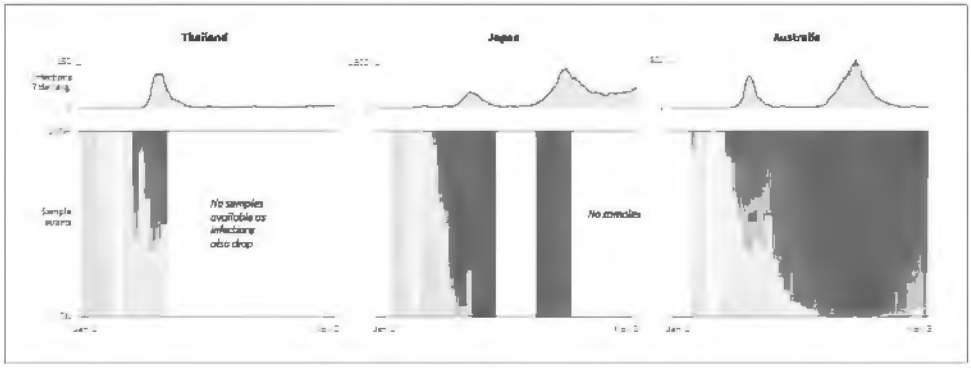
China



G strains take over

G strains are now dominant around the world. One specific mutation, D614G, has become the most common variant. It is so named because one amino acid is changed from a D (aspartate) to a G (glycine) at the 614th position on the viral spike proteins, the structure that gives the virus its crown-like appearance.

The rise of the G strains coincided with spikes in outbreaks of the virus around the world, with a clutch of new cases allowing the strains to invade new areas. The dominance of the G strains is illustrated by the data for Australia, Japan and Thailand. During Australia's second wave of infections, G strains were present in almost all samples, indicating the country had effectively eliminated transmission of the earlier L and S strains through a series of social distancing measures. All of Australia's second wave clusters were sparked by people who had returned from overseas and breaches in quarantine.

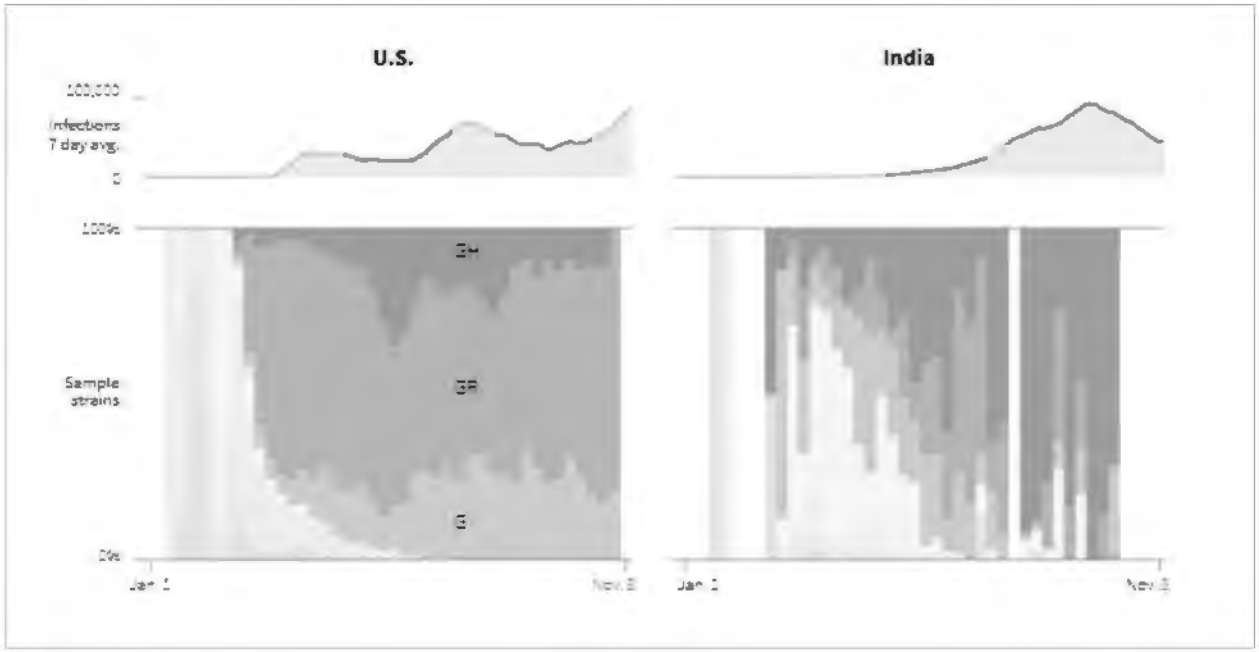


Major epicentres

The dominance of the G-strains becomes even more evident when looking at some countries with the most infections.

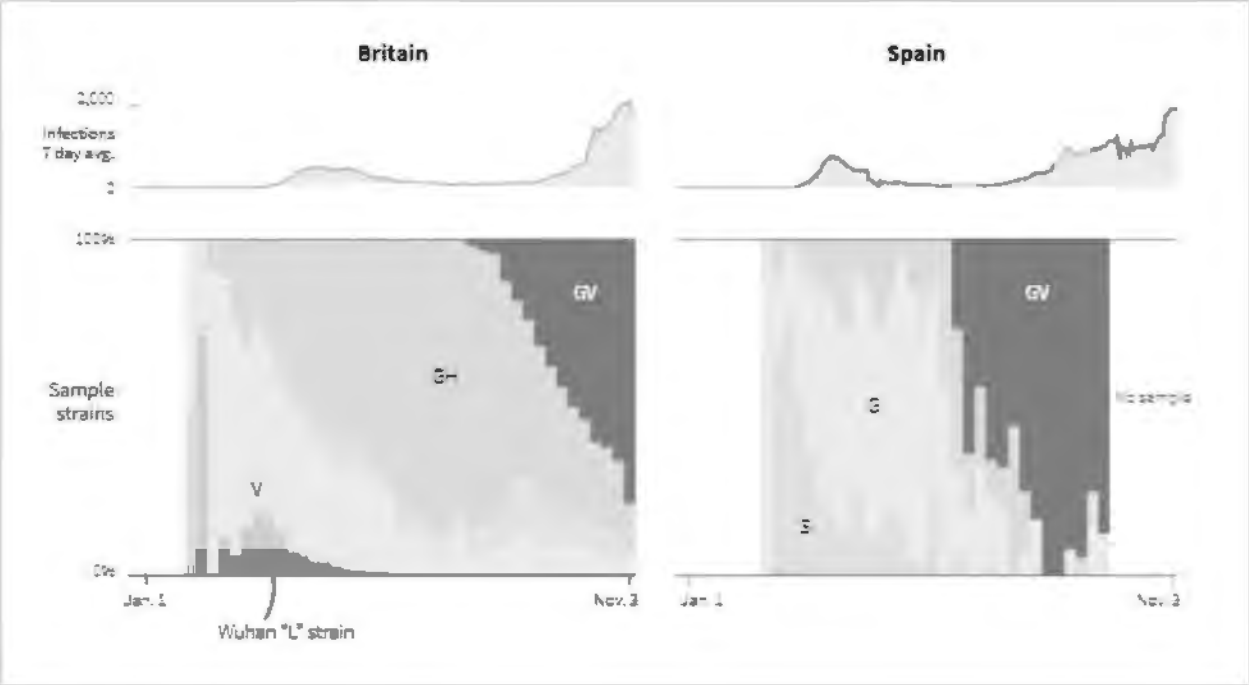
The United States is leading the overall number of infections and deaths by far. The majority of infections and first, second, and third waves all coincide with the increase in samples showing three G strains.

In India, a similar pattern can be observed as the constant increase in infections from June to September seemed to follow the curve of the G strain samples.



The new strain

The most recent mutation to emerge is the GV strain, which has so far been isolated to Europe where it has become increasingly common in recent weeks. GISAID scientists said the variant has a mutation in the protein spike, but in this case it may have little effect on the virus' ability to bind to human cells. Experts say it is currently unclear whether the GV strain is spreading because of any transmission advantage or because it affected socially active young adults and tourists over the summer.

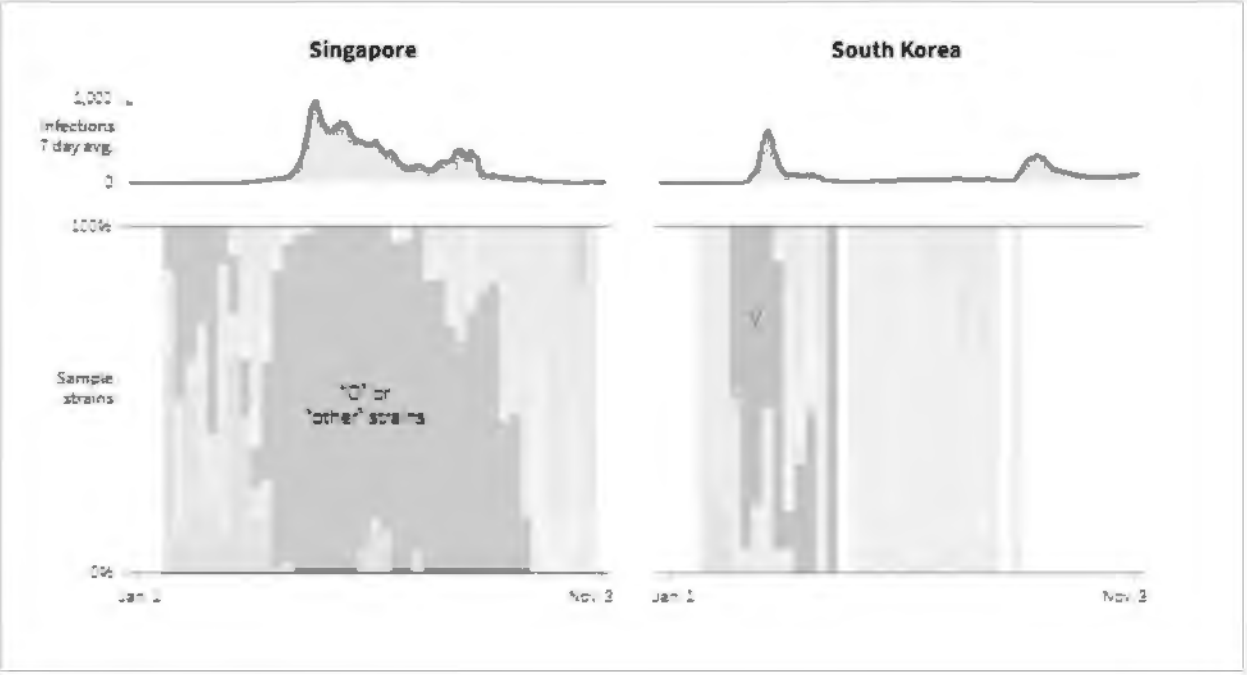


Outliers

Some countries bucked the general trend for a progression - albeit at varying rates - from the L to the G strains. In some cases, insufficient sample data was submitted to GISAID to detect a pattern. However, some other countries simply failed to follow the overarching shift to G-type strains.

Singapore, for example, recorded a significant number of O strains - virus variants that did not develop into sustained lineages - for several weeks. Deakin University's Bennett said that likely reflected the fact that most of Singapore's outbreaks were in separate foreign worker dormitories and quickly contained to those facilities.

In South Korea, the V strain became dominant for a period linked to a huge cluster of cases at a religious sect in the city of Daegu. South Korea is also at the centre of global efforts to research the potential of reinfection with a different strain of the virus after reports in April that scores of people who had recovered from COVID-19 later tested positive again. Health officials at the time said they suspected it was due to tests picking up remnants of the dead virus. Since then, there have been documented reports of individuals being reinfected with different versions of the virus. In a recently published paper in the journal Clinical Infectious Diseases, researchers from Seoul National University Hospital used computerised analysis to show that one woman was first infected with the V strain and later reinfected with a G strain.

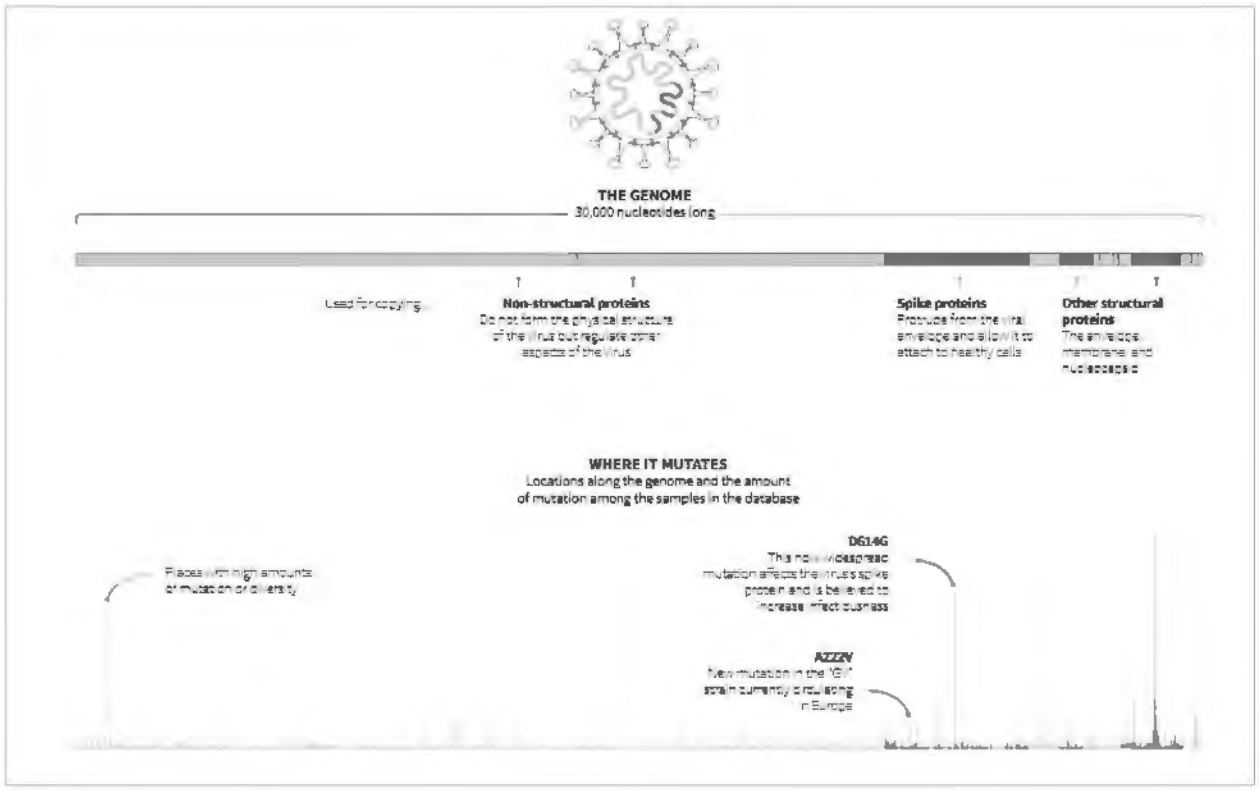


Why mutations matter

The mutations that give rise to new strains occur when the SARS-CoV-2 virus makes copies of itself inside a new host. The virus' genome is a complete set of genetic instructions that is written in 30,000 "letters" of code. Different sections of the genome guide how different parts of the virus, such as structural proteins of the shell or non-structural proteins that impact replication, are constructed when the virus replicates in host cells.

Small mutations in the virus's genome are normal as it is copied over and over. The GISAID database identified thousands of changes along the genome. Many are harmless but it's virtually impossible for scientists to predict when and how a mutation can result in a strain of a virus that is more transmissible or impervious to proposed vaccines.

The diagram below shows the various regions of the viral genome and the corresponding parts of the virus they encode, as well as the many mutations recorded in each genome region.



Cautious optimism

The SARS-CoV-2 virus has so far mutated slowly, allowing scientists and policy makers to keep on top of its progress. Still, scientists have been divided on the implications of some of the mutations. Some experts have reported that the D614G variation has made the virus more transmissible, however other studies have contradicted that.

Either way, the changes so far have not resulted in strains that would likely be resistant to vaccines in development. In fact, one study by a group of scientists from several institutions including the University of Sheffield and Harvard University found that G strains might present an easier target for a vaccine because these strains have more spike proteins on their surface, which are the target of vaccine-induced antibodies.

"Fortunately, we found that none of these mutations are making COVID-19 spread more rapidly, but we need to remain vigilant and continue monitoring new mutations, particularly as vaccines get rolled out," said University College of London Genetics Institute researcher Lucy van Dorp, co-author of a study that identified more than 12,700 mutations in the SARS-CoV-2 virus.

Still, experts who have watched influenza and HIV mutate over years, evading vaccines, warn that future mutations of SARS-CoV-2 remain unknown. And the best shot at avoiding changes that make the virus impervious to a vaccine remains curtailing its spread and reducing the opportunities it has to mutate.

"If the virus changes substantially, particularly the spike proteins, then it might escape a vaccine. We want to slow transmission globally to slow the clock," said Deakin's Bennett. "That reduces the chances of a one in a squillion change that's awful news for us."

Sources:
GISAID

By Jitesh Chowdhury, Simon Scarr and Jane Wardell
Editing by Christine Soares and Tiffany Wu

https://graphics.reuters.com/HEALTH-CORONAVIRUS/EVOLUTION/yxmpjqkdzvr/index.html

United States of America

Confidence Building Measure Return covering 2019

Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction

Submitted to the United Nations on
April 15, 2020

Declaration form on Nothing to Declare or Nothing New to Declare for use in the information exchange

Measure	Nothing to declare	Nothing new to declare	Year of last declaration if nothing new to declare
A, part 1			
A, part 2 (i)			
A, part 2 (ii)			
A, part 2 (iii)			
B			
C			
E			
F		√	1997
G			

Date: April 15, 2020

State Party to the Convention: United States of America

Date of ratification/accession to the Convention: March 26, 1975

National point of contact: Mr. Christopher Park, Department of State

Inquiries may be directed to ISN-BPS-DL@state.gov.

Report of the United States of America to the United Nations Department for Disarmament Affairs

Pursuant to the procedural modalities agreed upon in April 1987 at the "Ad Hoc Meeting of Scientific and Technical Experts for States Parties to the Convention on the Prohibition of the Development, Production, and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction," the United States of America submits the following information under Article V of the Convention:

BWC - Confidence Building Measure

Exchange of data on research centres and laboratories

United States of America

April 15, 2020

Form A, Part I (i)

Exchange of data on research centres and laboratories

1. Name(s) of facility.
National Biodefense Analysis and Countermeasures Center (NBACC)

2. Responsible public or private organization or company.
U.S. Department of Homeland Security Science and Technology Directorate
Operated by Battelle National Biodefense Institute LLC

3. Location and postal address.
8300 Research Plaza, Fort Detrick, Maryland 21702

4. Source(s) of financing of the reported activity, including indication if the activity is wholly or partly financed by the Ministry of Defence.
U.S. Department of Homeland Security (DHS)
U.S. Department of Justice (DOJ)
U.S. Department of Health and Human Services (HHS)

5. Number of maximum containment units within the research centre and/or laboratory, with an indication of their respective size (m^2).
BSL 4 Laboratory 980 m^2

6. Scope and general description of activities, including type(s) of microorganisms and/or toxins as appropriate.
NBACC conducts studies to better understand current and future biological threats; to assess vulnerabilities; and to determine potential impacts to guide the development of biological countermeasures such as detectors, drugs, vaccines, and decontamination technologies. When needed, NBACC conducts experimental programs to better characterize the benefits and risks of changes in U.S. biodefense preparations. NBACC also develops bioforensic assays and provides operational bioforensic analysis to support the attribution of biocrime and bioterrorism. (http://bnbi.org/)

The types of agents registered for use at NBACC are BSL-2 toxins, BSL-2 gram positive and gram negative bacterial agents, BSL-2 viral agents, BSL-3 gram positive and gram negative bacterial agents, BSL-3 viral agents, and BSL-4 viral agents.

Form A, Part I (i)

Exchange of data on research centres and laboratories

1. Name(s) of facility.
U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID)

2. Responsible public or private organization or company.
U.S. Army Medical Research and Materiel Command

3. Location and postal address.
1425 Porter Street, Fort Detrick, Frederick, Maryland 21702-5011

4. Source(s) of financing of the reported activity, including indication if the activity is wholly or partly financed by the Ministry of Defence.
U.S. Department of Defense (DoD) – Partly
U.S. Department of Homeland Security (DHS)
U.S. Department of Health and Human Services (DHHS)
U.S. Department of Agriculture (USDA)
U.S. Department of Energy (DoE)
U.S. Food and Drug Administration (FDA)
Universities
Private sector companies

5. Number of maximum containment units[3] within the research centre and/or laboratory, with an indication of their respective size (m^2).
BSL 4 Laboratory 1186 m^2

6. Scope and general description of activities, including type(s) of microorganisms and/or toxins as appropriate.
USAMRIID conducts research to develop strategies, products, information, procedures and training programs for medical defense against biological warfare threats and infectious diseases. Medical products developed to protect military personnel against biological agents include vaccines, drugs, diagnostic capabilities and various medical management procedures.

Additional information can be found at: http://www.usamriid.army.mil/

Form A, Part I (i)

Exchange of data on research centres and laboratories

1. Name(s) of facility.
Centers for Disease Control (CDC), Deputy Director for Infectious Disease (DDID)

2. Responsible public or private organization or company.
Centers for Disease Control and Prevention (CDC), Department of Health and Human Services (HHS)

3. Location and postal address.
1600 Clifton Road N.E., Atlanta, Georgia, 30329

4. Source(s) of financing of the reported activity, including indication if the activity is wholly or partly financed by the Ministry of Defence.
U.S. Department of Health and Human Services (HHS)

5. Number of maximum containment units[3] within the research centre and/or laboratory, with an indication of their respective size (m^2).
BSL-4 Laboratory 127 m^2
BSL-4 Laboratory 279 m^2
BSL-4 Laboratory 127 m^2

6. Scope and general description of activities, including type(s) of microorganisms and/or toxins as appropriate.
Activities include developing diagnostic assays for public health, developing and validating methods to differentiate and characterize organisms and the toxins that they produce, developing environmental sampling methods for recovery of agents from porous and nonporous surfaces for public health, routine reference antimicrobial susceptibility testing of clinical isolates, conducting molecular and antigenic characterization of organisms, determining pathogenicity and virulence of infectious agents, development of culture-independent point of care diagnostics, maintaining emergency response laboratory expertise and capacity, evaluating vaccines and medical countermeasures, determining the natural history of infectious organisms, assessing immune correlates of protection, and conducting epidemiologic studies and surveillance for diseases. Additional information can be found at: https://www.cdc.gov/ddid/. Please note, in 2019, 127 square meters of the reported BSL-4 space was utilized as BSL-3 Enhanced (BSL-3E) space, but remained capable of being used as BSL-4 space.

Biodefense activities include those with select agents (the select agents list is available at: http://www.selectagents.gov/SelectAgentsandToxinsList.html).

Form A, Part I (i)

Exchange of data on research centres and laboratories

1. Name(s) of facility
Integrated Research Facility at Fort Detrick (IRF – Frederick)

2. Responsible public or private organization or company
National Institutes of Health, Department of Health and Human Services
Operated by Battelle Memorial Institute

3. Location and postal address
8200 Research Plaza, Frederick, Maryland 21702

4. Source(s) of financing of the reported activity, including indication if the activity is wholly or partly financed by the Ministry of Defence
Department of Health and Human Services (HHS)

5. Number of maximum containment units[3] within the research centre and/or laboratory, with an indication of their respective size (m^2)
BSL-4 Laboratory 1305 m^2

6. Scope and general description of activities, including type(s) of micro-organisms and/or toxins as appropriate
The Integrated Research Facility at Fort Detrick in Frederick, Maryland (IRF-Frederick) is a component of the Division of Clinical Research of the National Institute of Allergy and Infectious Diseases (NIAID) at the National Institutes of Health (NIH). The mission of the IRF-Frederick is to manage, coordinate, and facilitate the conduct of emerging infectious disease and biodefense research to develop vaccines, countermeasures, and improved medical outcomes for patients. Research emphasis is placed on elucidating the nature of high consequence pathogens. Additional information can be found at: https://www.niaid.nih.gov/about/dir.

Form A, Part I (i)

Exchange of data on research centres and laboratories

1. Name(s) of facility
Integrated Research Facility at Rocky Mountain Laboratories (IRF-RML)

2. Responsible public or private organization or company
National Institutes of Health (NIH), Department of Health and Human Services (HHS)

3. Location and postal address
903 South 4th Street, Hamilton, Montana 59840 United States

4. Source(s) of financing of the reported activity, including indication if the activity is wholly or partly financed by the Ministry of Defence
Department of Health and Human Services (HHS)

5. Number of maximum containment units[3] within the research centre and/or laboratory, with an indication of their respective size (m^2)
BSL-4 Laboratory 1145 m^2

6. Scope and general description of activities, including type(s) of micro-organisms and/or toxins as appropriate
Rocky Mountain Laboratories (RML) is a component of the Division of Intramural Research of the National Institute of Allergy and Infectious Diseases (NIAID) at the National Institutes of Health (NIH). The RML mission is to play a leading role in the nation's efforts to develop diagnostics, vaccines, and therapeutics to combat emerging and re-emerging infectious diseases. Research at the Integrated Research Facility at Rocky Mountain Laboratories (IRF-RML) is dedicated to understanding the mechanisms of pathogenesis of microbial agents associated with or likely to cause serious or lethal human diseases using molecular methods and animal model systems. Additional information can be found at: https://www.niaid.nih.gov/about/rocky-mountain-laboratories.

Form A, Part I (i)

Exchange of data on research centres and laboratories

1. Name(s) of facility
Galveston National Laboratory (GNL) Complex including Robert E. Shope Laboratory

2. Responsible public or private organization or company
The University of Texas Medical Branch

3. **Location and postal address**
301 University Boulevard, Galveston, Texas 77555

4. Source(s) of financing of the reported activity, including indication if the activity is wholly or partly financed by the Ministry of Defence
State of Texas and the University of Texas Medical Branch
U.S. Department of Agriculture (USDA)
Private Foundations
Pharmaceutical and Biotechnology Industries
U.S. Department of Energy (DOE)
U.S. Department of Defense (DOD)
U.S. Department of Homeland Security (DHS)
National Institutes of Health (NIH)
Centers for Disease Control and Prevention (CDC)

5. Number of maximum containment units within the research centre and/or laboratory, with an indication of their respective size (m^2)
BSL-4 Laboratory 186 m^2 (Shope Laboratory)
BSL-4 Laboratory 1022 m^2 (GNL Laboratory)

6. Scope and general description of activities, including type(s) of microorganisms and/or toxins as appropriate
The mission of the Galveston National Laboratory is to assist the National Institute of Allergy and Infectious Diseases and the nation in the development of an improved means for the prevention, diagnosis and treatment of potentially life-threatening diseases caused by naturally emerging and purposefully disseminated infectious agents. To accomplish this goal GNL conducts multidisciplinary research into the causes, modes of transmission, and mechanisms of infectious diseases. Studies focus on a number of pathogens requiring BSL-4 containment, primarily those that cause viral hemorrhagic fevers, as well as some zoonotic viruses requiring enhanced BSL-3 containment. Products likely to emerge from research and investigations within the GNL include novel diagnostic assays, improved therapeutics and treatment models, and preventative measures such as vaccines.
Additional information can be found at: http://www.utmb.edu/gnl/.

Form A, Part I (i)

Exchange of data on research centres and laboratories

1. Name(s) of facility
The Betty Slick and Lewis J. Moorman, Jr. Laboratory Complex

2. Responsible public or private organization or company
Texas Biomedical Research Institute

3. Location and postal address
P.O. Box 760549, San Antonio, Texas 78245-0549

4. Source(s) of financing of the reported activity, including indication if the activity is wholly or partly financed by the Ministry of Defence
Department of Health and Human Services (HHS)
Department of Defense (DOD) - partly
Department of Homeland Security (DHS)
Private Sector Companies
Private Donors

5. Number of maximum containment units within the research centre and/or laboratory, with an indication of their respective size (m^2)
BSL 4 Laboratory 114 m^2

6. Scope and general description of activities, including type(s) of microorganisms and/or toxins as appropriate.
The mission of the Laboratory is to develop vaccines and therapeutics against viral pathogens, and to determine how viruses replicate and spread. Scientists are studying new and emerging disease threats, possible bioterrorism agents, and as-yet uncharacterized agents for biodefense. TXBiomed (formerly Southwest Foundation for Biomedical Research) has permits from the U.S. Department of Agriculture and the Centers for Disease Control to work on select agents. Additional information can be found at: https://www.txbiomed.org/about/extraordinary-resources/.

Form A, Part 1 (i)

Exchange of data on research centres and laboratories

1. Name(s) of facility
Georgia State University - High Containment Core (HCC)

(b)(5)

2. Responsible public or private organization or company
Georgia State University

3. Location and postal address
P.O. Box 4010, Atlanta, Georgia 30302-4118

4. Source(s) of financing of the reported activity, including indication if the activity is wholly or partly financed by the Ministry of Defence
National Institutes of Health
U.S. Department of Defense - partially
Centers for Disease Control and Prevention
Department of Health and Human Services
Georgia Research Alliance
Elizabeth R. Griffin Research Foundation

This facility resumed operation October 2019; agents (see question #6) are currently being stored in the facility. Active experimentation will not begin until July 2020. Funds listed above will be utilized at that time.

5. Number of maximum containment units within the research centre and/or laboratory, with an indication of their respective size (m^2)
BSL-4 60 m^2

6. Scope and general description of activities, including type(s) of microorganisms and/or toxins as appropriate

In 2017, the high containment facilities at Georgia State University were organized into the High Containment Core (HCC). The former National B Virus Resource Laboratory now operates as part of the core. The core is comprised of two BSL3 laboratories with animal facilities and one BSL4 Class III Cabinet Line Laboratory. Research in the BSL4 is focused on existing and emerging infectious diseases caused by BSL-4 viruses. The laboratory has not been used for experimental work since decommission in 2016. The facility was recommissioned in 2019 and was approved for storage of Tier 1 Select Agents and Toxins by the Centers for Disease Control and Prevention, Federal Select Agent Program. Experimental work with Risk Group 4 agents will commence in 2020. Below is a general description of those activities.

The proposed studies will expand our understanding of the mechanisms that regulate filovirus growth and pathogenesis. The goal is to characterize the impact of host proteins and genes on filovirus growth. We are specifically planning to perform these experiments with the filoviruses to mechanistically understand how different host factors affect virus replication, it will be necessary to measure levels of viral genomic RNA, viral mRNA and viral protein produced in cells.

Studies focused on the mechanisms by which virus kills the host and how that process can be circumvented with:

- Early identification - research focuses on the design and development of new approaches to more effectively identify these agents in both natural and foreign hosts;
- Appropriate antiviral drugs - researchers continually screen the efficacy of existing as well as novel antiviral agents to inhibit the growth of viruses that can potentially cross into the human population, either through occupational exposure or through more subtle contact; and
- In the future, effective vaccines. Additional information can be found at http://www2.gsu.edukwwwvir/Research/Index.html

Form A, Part I (i)

Exchange of data on research centres and laboratories

1. Name(s) of facility.
The Boston University National Emerging Infectious Diseases Laboratories (NEIDL)

2. Responsible public or private organization or company:
Boston University

3. Location and postal address.
620 Albany Street, Boston, MA 02118

4. Source(s) of financing of the reported activity, including indication if the activity is wholly or partly financed by the Ministry of Defence.
National Institute of Allergy and Infectious Disease (NIAID), U.S. National Institute of Health (NIH)
Boston University
U.S. Department of Health and Human Services (DHHS)
Pharmaceutical and Biotechnology companies
Private foundations

5. Number of maximum containment units[3] within the research centre and/or laboratory, with an indication of their respective size (m^2).

BSL-2 Laboratory	2,566 m^2
BSL-3 Laboratory	(5 suites + 8 auimal rooms) 998 m^2
BSL-4 Laboratory	(All ABSL-4 spaces are integrated with 6 suites + 7 animal rooms) 1,202 m^2

Note: The chauges in laboratory space from those reported in 2019 (BSL-2: 2,470 to 2,556 m^2; BSL-3: 960 to 998 m^2; BSL-4 968 to 1,202 m^2) were due to a numerical calculation error. The physical laboratory space remains unchanged.

6. Scope and general description of activities, including type(s) of microorganisms and/or toxins as appropriate.

The mission of the Boston University National Emerging Infectious Diseases Laboratories (NEIDL) is to generate and translate fundamental knowledge on high priority emerging infectious diseases for the benefit of the public health, locally, nationally, and globally. Emerging infectious diseases are defined as those that have newly appeared and been recognized in the population, or have existed but are rapidly increasing in incidence or in geographic range. To meet this mission the NEIDL will:

1. Perform innovative basic, translational, and clinical research on emerging infectious diseases, especially those identified as high priority category A, B, and C agents (http://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens), in order to develop diagnostics tests, treatments and vaccines to promote public health.
2. Provide education and training in these areas of research, in order to develop the next generation ofscientists in this field, and to support a national response in the event of a biodefense emergency.
3. Establish a research facility with the highest attention to community and laboratory safety and security.

Types of microorganisms currently being used are BSL-4 viruses. Additional informatiou can be found at: http://www.bu.edu/today/2017/neidl-bsl-4-lab-approved/

Form A. Part 2 (i)

BWC - Confidence Building Measure

National biological defence research and development programmes - Declaration

United States of America

April 15. 2020

Form A, Part 2 (i)

National biological defence research and development programme: Declaration

Are there any national programmes to conduct biological defence research and development within the territory of the State Party, under its jurisdiction or control anywhere? Activities of such programmes would include prophylaxis, studies on pathogenicity and virulence, diagnostic techniques, aerobiology, detection, treatment, toxinology, physical protection, decontamination and other related research.

Yes	X
No	

If the answer is Yes, complete Form A, part 2 (ii) which will provide a description of each programme

Form A, Part 2 (ii)

BWC - Confidence Building Measure

National biological defence research and development programmes - Description

United States of America

April 15, 2020

Form A, Part 2 (ii)

National biological defence research and development programmes: Overview

On September 18, 2018, the United States issued the National Biodefense Strategy, which contains goals and objectives that will guide the United States in assessing, preventing, detecting, preparing for, responding to, and recovering from a biological incident, whether deliberate, naturally occurring, or accidental in origin, and the accompanying Presidential Memorandum on Support for National Biodefense (NSPM-14) (see https://www.phe.gov/Preparedness/biodefense-strategy/Pages/default.aspx and https://www.whitehouse.gov/presidential-actions/presidential-memorandum-support-national-biodefense/; see also Form A, Part 2 (ii) and Form E). Integral to the strategy is a variety of research and development programs aimed at protecting against the deliberate use of biological materials to cause harm. These programs focus on the identification of harmful pathogens and outbreaks of infectious diseases, and their containment, treatment, and elimination from the environment. The programs are managed by several agencies with direct stakes in national security, environmental protection, and human and animal health and safety, including the Departments of Agriculture, Defense, Energy, Health and Human Services, Homeland Security, and the Environmental Protection Agency.

Historically, certain pathogens were selected for use as biological weapons because of their pathogenicity and other characteristics. Research on these pathogens, including study of molecular mechanisms and related diagnostic, vaccine and therapeutic development work, not only increases U.S. biodefense preparedness, but also offers inherent benefits for broader public health needs. Efforts to improve medical product stability, potency and ease-of-use that cut across disease targets could yield significant benefits for public health systems that cannot support existing treatments that require refrigeration, multiple doses or sophisticated diagnostic techniques. Similarly, biodefense initiatives to improve human and animal host defenses, to monitor emerging infectious diseases and drug-resistant microbes, and to clean up the site of a biological weapons attack benefit public health services, such as epidemiological disease surveillance and environmental remediation.

To promote the benefits gained by these programs and to ensure that the research is available to the scientific community both domestically and internationally, the United States Government encourages the publication of research funded by its biodefense programs.

For more information on U.S. Government strategies related to biodefense, including biological threat preparedness and response, please consult:

- Management of Domestic Incidents (Homeland Security Presidential Directive 5 [HSPD-5]) and the related National Response Framework;
- Presidential Policy Directive 8: National Preparedness (PPD-8);
- National Strategy for Defense of United States Agriculture and Food (HSPD-9);
- Medical Countermeasures against Weapons of Mass Destruction (HSPD-18);
- Public Health and Medical Preparedness (HSPD-21);
- Executive Order 13527 ("Establishing Federal Capabilities for the Timely Provision of Medical Countermeasures following a Biological Attack").

(b)(5)

Form A, Part 2 (ii)

National biological defence research and development programmes:

Description

1. State the objectives and funding of each programme and summarize the principal research and development activities conducted in the programme. Areas to be addressed shall include: prophylaxis, studies on pathogenicity and virulence, diagnostic techniques, aerobiology, detection, treatment, toxinology, physical protection, decontamination and other related research.

The Department of Defense Chemical and Biological Defense Program develops capabilities to enable the U.S. Armed Forces to deter, prevent, protect from, mitigate, respond to, and recover from the effects of chemical, biological, and radiological (CBR) threats as part of a layered, integrated defense. The Program is an integral contributor to a global and systems approach for Countering Weapons of Mass Destruction (CWMD), Global Health Security, and other pertinent mission areas.

The Program works to counter biological threats by providing complementary sets of sensors, protective equipment, and medical countermeasures to counter known and unknown threats, including novel and naturally-occurring emerging infectious diseases that may also pose a biological weapons threat. Current research focuses on host-pathogen interactions; capabilities for pre- and post-exposure therapeutics for bacterial biological select agents and novel threats; testing battlefield detection and identification methods, protective systems, and decontamination systems; the development of rapid and deployable detection assays for troop protection; and medical defenses against neurotoxins.

The Program also works on producing self-disinfecting and/or self-decontaminating materials, as well as developing, producing, and fielding capabilities for sampling, detecting, and identifying biological agents.

Biological defense related work conducted by the Department of Defense is carried out by the military services and biological defense program-focused agencies. These include funding agencies and service laboratories within the Departments of the Air Force, Army, and Navy, and the Defense Threat Reduction Agency/Joint Science and Technology Office, the Joint Program Executive Office for Chemical and Biological Defense, and the Defense Advanced Research Projects Agency.

2. State the total funding for each programme and its source.
$530,565,000 U.S. Department of Defense (DOD)

3. Are aspects of these programmes conducted under contract with industry, academic institutions, or in other non-defence facilities?
Yes

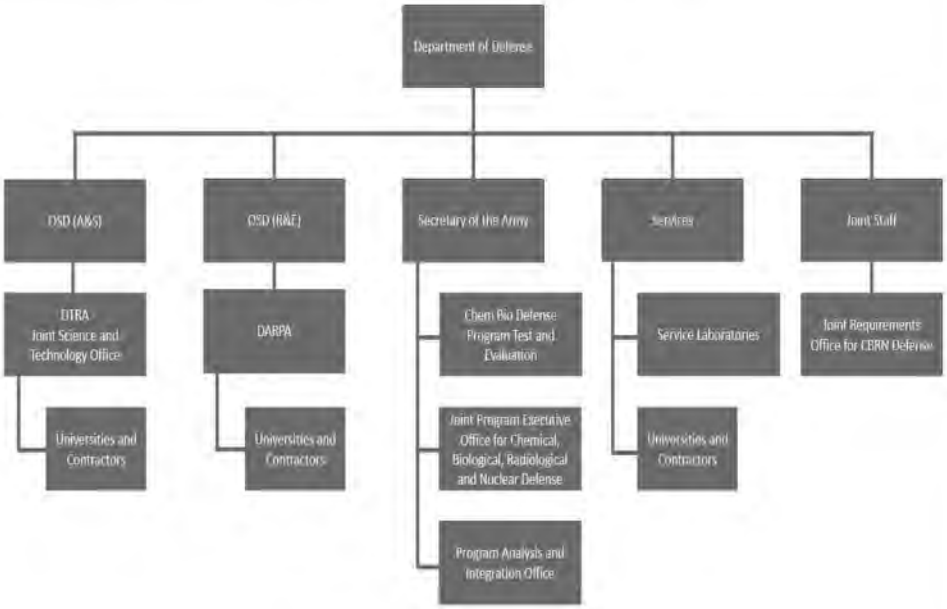
4. If yes, what proportion of the total funds for each programme is expended in these contracted or other facilities?
58.04%

5. Summarize the objectives and research areas of each programme performed by contractors and in other facilities with the funds identified under paragraph 4.

- Provide support and capabilities to protect the U.S. Armed Forces against biological warfare threats
- Development, testing, and manufacturing of vaccines, therapeutics, and diagnostic systems
- Development of self-disinfecting and/or self-decontaminating materials

- Development and testing of detection and identification methods, protective equipment, and decontamination systems

6. Provide a diagram of the organizational structure of each programme and the reporting relationships (include individual facilities participating in the programme).



This chart reflects funding relationships

7. Provide a declaration in accordance with Form A, part 2 (iii) for each facility, both governmental and non-governmental, which has a substantial proportion of its resources devoted to each national biological defence research and development programme, within the territory of the reporting State, or under its jurisdiction or control anywhere.

- Naval Medical Research Center (NMRC) – Page 47
- Naval Research Laboratory (NRL) – Page 50
- Naval Surface Warfare Center-Dahlgren Division Chemical, Biological, Radiological (CBR) Defense Laboratory – Page 53
- Lothar Salomon Life Sciences Test Facility (LSTF) – Page 45
- U.S. Army Combat Capabilities Development Command Chemical Biological Center (CCDC CBC), formerly named U.S. Army Edgewood Chemical and Biological Center – Page 55
- U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) – Page 58
- U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) – Page 60

Neither the U.S. Army Combat Capabilities Development Command Soldier Center (CCDC SC), formerly named U.S. Army Natick Soldier Research Development and Engineering, nor the Air Force

Research Laboratory (AFRL), received funding for biodefense work in 2019 and are not included in the 2019 Confidence Building Measures.

(b)(5)

Form A, Part 2 (ii)

National biological defense research and development programmes:

Description

1. State the objectives and funding of the programme and summarize the principal research and development activities conducted in the programme. Areas to be addressed shall include: prophylaxis, studies on pathogenicity and virulence, diagnostic techniques, aerobiology, detection, treatment, toxicology, physical protection, decontamination and other related research.

The Environmental Protection Agency (EPA)'s mission is to protect public health and the environment. The Homeland Security Research Program (HSRP), part of the EPA's Office of Research and Development, conducts and reports on research to improve capacity to respond to and recover from environmental contamination of water infrastructure, buildings, and outdoor areas by chemical, biological, radiological and nuclear (CBRN) agents. The HSRP biodefense program focuses on EPA's two biodefense responsibilities: 1) assistance in the protection of the American water supply, and 2) decontamination of indoor and outdoor areas should the U.S. suffer a contamination incident.

EPA is designated as the government's lead sector-specific agency for water and is responsible for protecting water systems and detecting and recovering from terrorist attacks affecting them. EPA's homeland security research is responsible for developing products and providing expertise to protect, detect, respond to, and recover from terrorist attacks on the nation's water and wastewater infrastructure.

EPA is also the lead federal agency for the remediation of areas contaminated by terrorist events involving the release of biological organisms, biotoxins, chemical warfare agents, toxic industrial chemicals, and radiological materials. Terrorist acts may involve biological, chemical, and radiological agents not previously encountered as environmental pollutants. EPA's homeland security research is responsible for providing procedures and methods that will assist EPA's responders in the characterization and containment of contamination, and in the remediation of sites following terrorist attacks.

2. State the total funding for the programme and its source.
$7,600,000 U.S. Environmental Protection Agency (EPA)

3. Are aspects of the programme conducted under contract with industry, academic institutions, or in other non-defense facilities?
Yes

4. If yes, what proportion of the total funds for the programme is expended in these contracted or other facilities?
50%

5. Summarize the objectives and research areas of the programme performed by contractors and in other facilities with the funds identified in paragraph 4.
To address capabilities related to EPA's indoor/outdoor remediation mission, HSRP, through intramural and extramural avenues, conducts research related to characterization methods, risk assessment, decontamination methods, and waste management. Specifically, the program develops and evaluates 1) sampling and analytical methods for environmental matrices, 2) decontamination methods for complex environments, and 3) treatment methods for solid and liquid waste. Supporting such capabilities, HSRP

has been addressing the fate and transport of biological agents and developing exposure assessment information and methods to support risk assessment decisions.

6. Provide a diagram of the organizational structure of the programme and the reporting relationships (include individual facilities participating in this programme.)

Note: EPA's Office of Research and Development underwent organizational structural changes, effective in September 2019.

(b)(5)

7. Provide a declaration in accordance with Form A part 2 (iii) for each facility, both governmental and non-governmental, which has a substantial proportion of its resources devoted to the national biological defense research programme, within the territory of the reporting State, or under its jurisdiction or control anywhere.

Not Applicable

Form A, Part 2 (ii)

National biological defence research and development programmes:

Description

1. State the objectives and funding of each programme and summarize the principal research and development activities conducted in the programme. Areas to be addressed shall include: prophylaxis, studies on pathogenicity and virulence, diagnostic techniques, aerobiology, detection, treatment, toxicology, physical protection, decontamination and other related research.

The Department of Health and Human Services (HHS) supports activities to improve local and state public health systems, to expand existing biosurveillance efforts, and to fund research on medical countermeasures against potential bioterror agents.

The National Institutes of Health (NIH) biodefense program is supported by funding from HHS. The NIH, and specifically the National Institute of Allergy and Infectious Diseases (NIAID), has the primary responsibility within the U.S. Government for civilian biodefense research. The intent of the program is to provide countermeasures to be used to protect the U.S. civilian population through the development of vaccines, therapeutic agents and rapid, agent-specific assays.

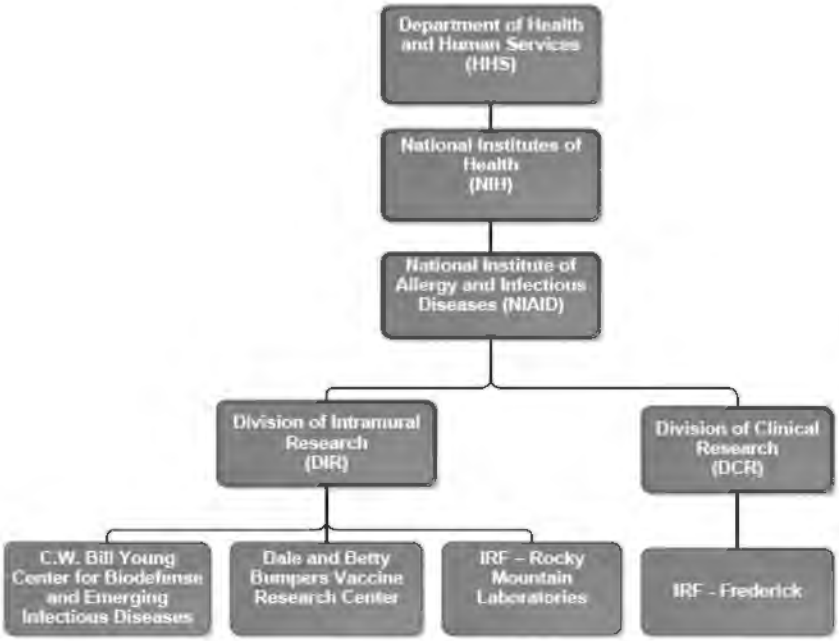
2. State the total funding for each programme and its source.
$87,671,966 Department of Health and Human Services (HHS)

3. Are aspects of these programmes conducted under contract with industry, academic institutions, or in other non-defence facilities?
Yes

4. If yes, what proportion of the total funds for each programme is expended in these contracted or other facilities?
17.5%

5. Summarize the objectives and research areas of each programme performed by contractors and in other facilities with the funds identified under paragraph 4.
Battelle Memorial Institute facilitates scientific research at the Integrated Research Facility at Fort Detrick (IRF-Frederick), including refinement of animal models to facilitate countermeasure development, with direction from the IRF Scientific Steering Committee.

6. Provide a diagram of the organizational structure of each programme and the reporting relationships (include individual facilities participating in the programme).



7. Provide a declaration in accordance with Form A, part 2 (iii) for each facility, both governmental and non-governmental, which has a substantial proportion of its resources devoted to each national biological defence research and development programme, within the territory of the reporting State, or under its jurisdiction or control anywhere.

- Integrated Research Facility at Fort Detrick (IRF - Frederick) – Page 108
- Integrated Research Facility at Rocky Mountain Laboratories (IRF - RML) – Page 100
- C.W. Bill Young Center for Biodefense and Emerging Infectious Diseases – Page 112
- Dale and Betty Bumpers Vaccine Research Center – Page 122

Form A, Part 2 (ii)

National biological defence research and development programmes:

Description

1. State the objectives and funding of each programme and summarize the principal research and development activities conducted in the programme. Areas to be addressed shall include: prophylaxis, studies on pathogenicity and virulence, diagnostic techniques, aerobiology, detection, treatment, toxicology, physical protection, decontamination and other related research.

The objective of the Mass Spectrometry Toxin Laboratory and the Chemical Threats Method Development Laboratory within CDC's National Center for Environmental Health, Division of Laboratory Sciences is to develop methods for measuring selected toxins to help improve detection and diagnosis during a public health response to biological toxins.

2. State the total funding for each programme and its source.
$5,248,729.78 Centers for Disease Control and Prevention (CDC)

3. Are aspects of these programmes conducted under contract with industry, academic institutions, or in other non-defence facilities?
No

4. If yes, what proportion of the total funds for each programme is expended in these contracted or other facilities?
N/A

5. Summarize the objectives and research areas of each programme performed by contractors and in other facilities with the funds identified under paragraph 4.
N/A

6. Provide a diagram of the organizational structure of each programme and the reporting relationships (include individual facilities participating in the programme).

7. Provide a declaration in accordance with Form A, part 2 (iii) for each facility, both governmental and non-governmental, which has a substantial proportion of its resources devoted to each national biological defence research and development programme, within the territory of the reporting State, or under its jurisdiction or control anywhere.

CDC, National Center for Environmental Health (NCEH), Division of Laboratory Sciences (DLS) – Page 87

Form A, Part 2 (ii)

National biological defence research and development programmes:

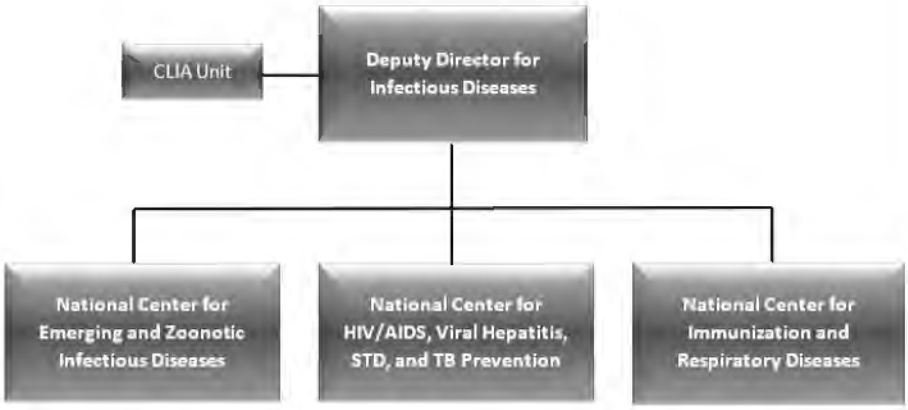
Description

1. **State the objectives and funding of each programme and summarize the principal research and development activities conducted in the programme. Areas to be addressed shall include: prophylaxis, studies on pathogenicity and virulence, diagnostic techniques, aerobiology, detection, treatment, toxinology, physical protection, decontamination and other related research.**

   The activities of the CDC Deputy Director for Infectious Disease (DDID) include developing diagnostic assays for public health, conducting molecular and antigenic characterization of microorganisms, evaluating decontamination methods, determining pathogenicity and virulence of infectious agents, determining the natural history of infectious organisms, and conducting epidemiologic studies and surveillance for diseases. Biodefense activities include those with select agents. DDID includes the National Center for Emerging Zoonotic Infectious Diseases (NCEZID) and the National Center for Immunization and Respiratory Diseases (NCIRD).

   The select agents list is available at: http://www.selectagents.gov/SelectAgentsandToxinsList.html

2. **State the total funding for each programme and its source.**
   $ 29,030,375 Centers for Disease Control and Prevention (CDC)
3. **Are aspects of these programmes conducted under contract with industry, academic institutions, or in other non-defence facilities?**
   Yes
4. **If yes, what proportion of the total funds for each programme is expended in these contracted or other facilities?**
   5%
5. **Summarize the objectives and research areas of each programme performed by contractors and in other facilities with the funds identified under paragraph 4.**
   Vaccine efficacy trials, reagent development, bioterrorism preparedness and response activities, avian influenza preparedness, and disease surveillance in CDC field locations.
6. **Provide a diagram of the organizational structure of each programme and the reporting relationships (include individual facilities participating in the programme).**



7. Provide a declaration in accordance with Form A, part 2 (iii) for each facility, both governmental and non-governmental, which has a substantial proportion of its resources devoted to each national biological defence research and development programme, within the territory of the reporting State, or under its jurisdiction or control anywhere.

- CDC, Deputy Director for Infectious Diseases (DDID) – Page 89
- CDC, Deputy Director for Infectious Diseases (DDID), National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Vector Borne Diseases (DVBD) - Ft. Collins – Page 98

Form A, Part 2 (ii)

National biological defence research and development programmes:

Description

1. State the objectives and funding of the programme and summarize the principal research and development activities conducted in the programme. Areas to be addressed shall include: prophylaxis, studies on pathogenicity and virulence, diagnostic techniques, aerobiology, detection, treatment, toxicology, physical protection, decontamination and other related research.

Background

The U.S. Department of Agriculture's Agricultural Research Service (USDA-ARS) biodefense research program addresses foreign pathogens of plants and animals that represent a major threat to U.S. agriculture. Introduction of these agents, either accidental or deliberate, could have devastating effects on animal or plant health, and in some cases, human health. These devastating effects extend to social and economic impacts -- not only in the country's agricultural systems but also in a wide range of economic activities. Diseases of concern include but are not limited to wheat rust, Foot-and-Mouth Disease, Avian Influenza, Rift Valley Fever, Classical Swine Fever, African Swine Fever, Exotic Newcastle disease, Vesicular stomatitis, and Exotic Bluetongue.

Plant and Animal health officials define an exotic or foreign plant or animal disease as important infectious diseases of crops, livestock or poultry believed to be absent from the U.S. and its territories that has a potential significant health or economic impact. In addition, zoonotic foreign animal diseases pose a threat to human health and animal production potentially resulting in appreciable costs due to expensive disease control and eradication efforts. To protect the long-term health and profitability of U.S. animal agriculture, incursions of a foreign animal disease must be rapidly controlled.

In the United States, control is the first step towards disease eradication. Disease eradication is currently accomplished by eliminating crops or animals, resulting in loss of foods, loss of income to the farm community, public opposition and environmental disruption. In addition to control costs, one of the most immediate and severe consequences of a foreign animal disease occurrence in the United States will be the loss of export markets. As we approach the third decade of the 21st century, many new issues and factors are affecting prevention, control, management, and recovery from foreign disease outbreaks. These factors include free trade agreements, free trade blocks, regionalization, increased international passenger travel, intensification of plant and animal production, increased climate instability, the constant evolution of infectious agents, and the uncertain impact of biotechnology and bioterrorism.

The USDA-ARS biodefense program focuses its research efforts on the prevention, detection, control, and eradication of high consequence foreign plant and animal diseases. Research efforts include furthering our understanding of pathogenesis, transmission, and host responses to emerging plant and animal diseases to enhance rapid detection and developing effective countermeasures.

Strategic Objectives

- Establish Agricultural Research Service (ARS) laboratories into a fluid, highly effective research network, to maximize the use of core competencies and resources
- Access specialized high containment research facilities to study zoonotic and emerging diseases
- Develop an integrated animal and microbial genomics research program

- Establish centers of excellence in animal immunology
- Launch a biotherapeutic discovery program providing alternatives to conventional animal drugs
- Build a technology-driven vaccine and diagnostic discovery research program
- Develop core competencies in field epidemiology and predictive biology
- Develop internationally recognized World Organisation for Animal Health (OIE) collaborative research centers
- Establish a best-in-class training center for our nation's veterinarians and scientists
- Develop a model technology transfer program to achieve the full impact of our research discoveries
- Determine basic knowledge of the biology, pathology, and epidemiology of selected plant Oomycete pathogens as the basis for development of improved control/management strategies

Research Needs

In order to control foreign animal disease, a wide variety of agent detection platforms needs to be developed and validated. Information for design of these platforms will come in part from further knowledge of pathogen genomics and proteomics and in part from understanding the evolution and genetic variability of disease agents. Although many of the foreign animal diseases have existed for many years in many countries, there is still much more fundamental knowledge of these agents that is required. There is still a lack of understanding in host range and tissue tropism, carrier state, duration and routes of shedding, transmission mechanisms, (e.g. vectors, fomites, aerosols), ecology and epidemiology (e.g., wildlife reservoirs). Lack of reagents, and the lack of stockpiling of diagnostic kits and supplies present vulnerabilities in detection and response preparedness. Effective prevention and control tools need to be developed in order to prepare for the possibility of a foreign animal disease outbreak in the United States. These could include tools for identifying suitable control strategies which take into account the short amount of time available and the cost of recovery from disease outbreaks. There is a need for development of vaccines and biotherapeutics suitable for strategic stockpiles and for integrated methods of disease control (including vector control and animal management), which lead to a better capability to regain country disease-free status and retain economic sustainability.

Expected Outputs:

- Better anticipation of introduction of foreign animal diseases (FADs)
- Capability to advise regulatory officials on scientific procedures for the prevention of introduction of FADs
- Better capability to produce effective products to control and eliminate FADs
- Real-time detection of agents in a wide range of farm matrices
- Searchable databases of genome and proteome information for major known FAD agents
- Improved ability to predict or anticipate emergence or introduction FAD agents
- Discovery of effective candidate biotherapeutics
- Discovery of effective candidate vaccines that allow differentiation of infected animals from vaccinated animals (DIVA)
- Viable integrated vector control strategies that minimize losses
- In-depth knowledge of pathogen biology, taxonomy, genetics, ecology, and pathology of emerging Oomycete pathogens that can be used to develop novel and effective exclusion, control and management strategies

The USDA-ARS biodefense research program is intramural and implemented in ARS high containment facilities in the following locations: Ames, Iowa; Orient Point, New York; Athens, Georgia; and Frederick, Maryland.

2. State the total funding for the programme and its source.

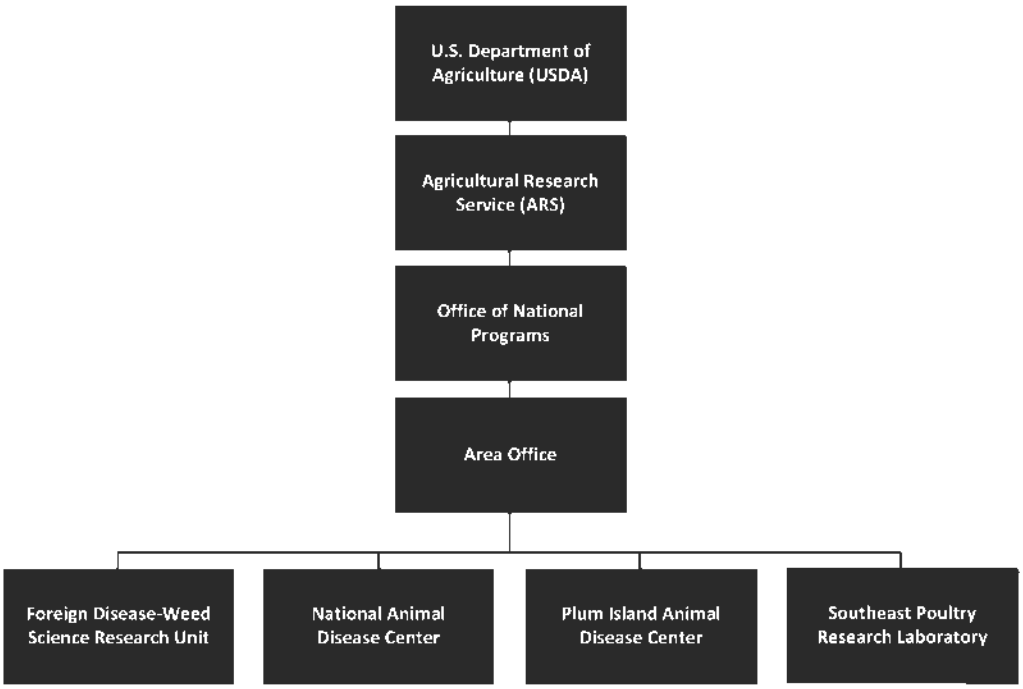
$24,000,000 U.S. Department of Agriculture (USDA)

3. Are aspects of the programme conducted under contract with industry, academic institutions, or in other non-defence facilities?
No

4. If yes, what proportion of the total funds for the programme is expended in these contracted or other facilities?
Not Applicable

5. Summarize the objectives and research areas of the programme performed by contractors and in other facilities with the funds identified in paragraph 4.
Not Applicable

6. Provide a diagram of the organizational structure of the programme and the reporting relationships (include individual facilities participating in this programme.)



7. Provide a declaration in accordance with Form A part 2 (iii) for each facility, both governmental and non-governmental, which has a substantial proportion of its resources devoted to the national biological defence research programme, within the territory of the reporting State, or under its jurisdiction or control anywhere.

- Foreign Disease-Weed Science Research Unit – Page 124
- National Animal Disease Center (NADC) – Page 126
- Southeast Poultry Research Laboratory – Page 129
- Plum Island Animal Disease Center (PIADC) – Page 41

Form A, Part 2 (ii)

National biological defence research and development programmes:

Description

1. **State the objectives and funding of the programme and summarize the principal research and development activities conducted in the programme. Areas to be addressed shall include: prophylaxis, studies on pathogenicity and virulence, diagnostic techniques, aerobiology, detection, treatment, toxicology, physical protection, decontamination and other related research.**

Preventing terrorism and enhancing security, including protection against biological terrorism, is one of the five key Department of Homeland Security (DHS) mission areas. This includes efforts to: prevent terrorist attacks, including biological attacks; prevent the unauthorized acquisition, importation, movement, or use of, *inter alia*, biological materials and capabilities within the United States; and reduce the vulnerability of critical infrastructure to terrorist attacks and other hazards. These efforts are further guided by the National Biodefense Strategy, which outlines five goals: enable risk awareness to inform decision-making across the biodefense enterprise; ensure biodefense enterprise capabilities to prevent bioincidents; ensure biodefense enterprise preparedness to reduce the impacts of bioincidents; rapidly respond to limit the impacts of bioincidents; and facilitate recovery to restore the community, the economy, and the environment after a bioincident.

The goal of the DHS biodefense program is to protect against biological attacks targeting the U.S. population, agriculture, or infrastructure. The DHS Biodefense program focuses on scenario modelling, agent release detection, training in responding to biological events, biological countermeasures research, development, testing, and evaluation (RDT&E) efforts, and on the transition of resultant technologies to operational use. The five main areas of study are: 1) systems studies and decision support tools, 2) threat awareness, 3) surveillance and detection research and development (R&D), 4) forensics, and 5) response and restoration. The program supports other U.S. federal agencies in overall coordination of national biodefense efforts.

Efforts conducted during 2019 included comprehensive threat and risk assessments to guide prioritization of the Nation's biodefense investments, biodefense knowledge management, the development of next-generation detectors for biological threat agents for critical infrastructure and urban areas, decontamination of transit systems, and bioforensics research in support of criminal investigations and attribution. Efforts at the National Biodefense Analysis and Countermeasures Center included biological threat characterization and forensic analysis for attribution, and, at the Plum Island Animal Disease Center, development of vaccines and diagnostics for foreign animal diseases.

The DHS Compliance Review Group, chaired by the DHS Deputy Secretary, met in 2019 to review all relevant DHS-funded biological defense projects for compliance with the provisions of the Biological Weapons Convention and associated U.S. domestic laws and policies.

2. **State the total funding for the programme and its source.**

$78,421,000 U.S. Department of Homeland Security (DHS)

3. **Are aspects of the programme conducted under contract with industry, academic institutions, or in other non-defence facilities?**

Yes

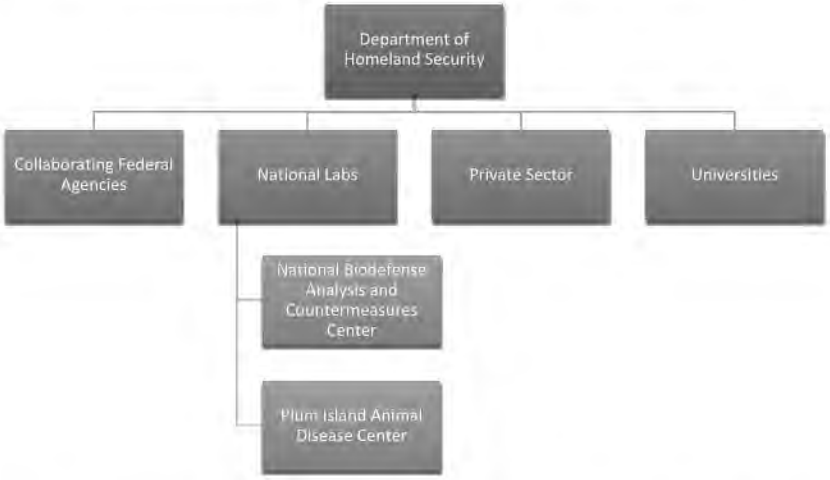
4. **If yes, what proportion of the total funds for the programme is expended in these contracted or other facilities?**

100%

5. **Summarize the objectives and research areas of the programme performed by contractors and in other facilities with the funds identified in paragraph 4.**

Identical to answer provided in question 1.

6. **Provide a diagram of the organizational structure of the programme and the reporting relationships (include individual facilities participating in this programme).**



7. **Provide a declaration in accordance with Form A part 2 (iii) for each facility, both governmental and non-governmental, which has a substantial proportion of its resources devoted to the national biological defence research programme, within the territory of the reporting State, or under its jurisdiction or control anywhere.**

- National Biodefense Analysis and Countermeasures Center (NBACC) – Page 38
- Plum Island Animal Disease Center (PIADC) – Page 41

Form A, Part 2 (iii)

BWC - Confidence Building Measure

National biological defence research and development programmes - Facilities

United States of America

April 15, 2020

Form A, Part 2 (iii)

National biological defence research and development programme

For each facility detailed on Form A, Part 2 (iii), the entries given for question 3, "Floor area of laboratory areas by containment level (m^2)" represent lab space used for biodefense R&D purposes during CY19. Year-to-year variations in programming may result in variations in laboratory space reported rather than alterations to the physical laboratory space.

The U.S. Government identified potential concerns associated with public release of information regarding the presence of highly pathogenic microorganisms and toxins at specific facilities. To balance these concerns with a desire to promote transparency, rather than listing the specific microorganisms and toxins at individual facilities, the U.S. public CBM return characterizes microorganisms and toxins studied at each facility on Form A, Part 2 (iii) simply as "Select Agents" and/or "NIAID Category A pathogens." The full lists of Select Agents and NIAID pathogens are found in Appendix A. Biological Select Agents and Toxins (Select Agents) are biological agents or toxins that have the potential to pose a severe threat to public, animal or plant health, or to animal or plant products. Possession, use and transfer of Select Agents are regulated by the Select Agent Rules. Detailed information on Select Agents and their regulation can be found at: http://www.selectagents.gov. The NIAID designated Category A pathogens as priorities for additional research efforts as part of the NIAID biodefense research agenda. Detailed information about NIAID Category A pathogens can be found at: http://www.niaid.nih.gov/topics/BiodefenseRelated/Biodefense/Pages/CatA.aspx.

The U.S. public CBM also includes an Appendix B, which is a combined list of all of the specific microorganisms and toxins studied for biodefense research and development at *all* facilities reported on Form A, part 2 (iii) below. To maintain a high level of transparency to States Parties, the United States makes available, via the restricted-access portion of the ISU website, a Supplement containing information on the microorganisms and toxins studied at each individual facility reported on Form A, part 2 (iii).

Form A, Part 2 (iii)

National biological defence research and development programmes: Facilities

1. What is the name of the facility?
National Biodefense Analysis and Countermeasures Center (NBACC)

2. Where is it located (provide both address and geographical location)?
8300 Research Plaza, Fort Detrick, Maryland 21702

3. Floor area of laboratory areas by containment level (m^2):

BSL-2:	1,307 m^2
BSL-3:	2,564 m^2
BSL-4:	980 m^2
Total laboratory floor area:	4,851 m^2

4. The organizational structure of each facility:

(i) Total number of personnel: 193

(ii) Division of personnel:

Military	0
Civilian	193

Division of personnel by category:

Scientists	40
Engineers	43
Technicians	62
Administrative and support staff	48

(iii) List the scientific disciplines represented in the scientific/engineering staff:
Aerobiology, Analytical Mass Spectrometry, Bacteriology, Biochemistry, Bioinformatics, Biological Science, Biomedical Science, Biophysics, Biotechnology, Cell Biology, Chemistry, Computer Science, Genetics, Genomics, Immunology, Microbial Forensics, Microbiology, Microscopy, Molecular Biology, Toxicology, Veterinary Medicine, Virology

(iv) Are contractor staff working in the facility? If so, provide an approximate number:
Yes Number: 193

(v) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
U.S. Department of Homeland Security (DHS)
U.S. Department of Justice (DoJ)
U.S. Department of Health and Human Services (HHS)

(vi) What are the funding levels for the following program areas:

Research	$ 9,604,849
Development	$ 14,049,050
Test and evaluation	$ 0
Total	$ 23,653,899

(vii) Briefly describe the publication policy of the facility:

The NBACC publication policy is to present research results to the greater scientific community as widely as possible. As a Federally Funded Research and Development Center (FFRDC) engaged in research with select agents/regulated pathogens, NBACC has established a formal, multi-tiered review system to ensure compliance and conformance with U.S. Government laws, regulations and policies including: export control regulations under Export Administration Regulations (EAR) and International Traffic in Arms Regulations (ITAR); the Biological Weapons Convention (BWC), and internal U.S. Department of Homeland Security (DHS) policies. Prior to submittal to journals or release, all publications are reviewed by NBACC and DHS for security, clarity, and accuracy with regard to the description of the work. The DHS Management Directive for Review of External Publications can be found at https://www.dhs.gov/sites/default/files/publications/mgmt/public-affairs/mgmt-dir_md-2260-1-review-of-external-publications.pdf.

(viii) Provide a list of publicly-available papers and reports resulting from the work during the previous 12 months. (To include authors, titles, and full references.):

1. Matthews L, Goodrich JS, Weber DJ, Bergman NH, Miller MB. Closing the Brief Case: A fatal case of necrotizing fasciitis due to multidrug-resistant Acinetobacter baumannii. J Clin Microbiol. 2019; 57(7):e01751-18. https://jcm.asm.org/content/57/7/e01754-18.long
2. Heller NC, Garrett AM, Merkley ED, Cendrowski SR, Melville AM, Arce JS, et al. Probabilistic Limit of Detection for Ricin Identification Using a Shotgun Proteomics Assay. Anal Chem. 2019; 91(19):12399−12406.https://pubs.acs.org/doi/10.1021/acs.analchem.9b02721
3. Cendrowski SR and Garrett AM. ISO 17025 Accreditation of Method-Based Mass Spectrometry for Bioforensic Analyses. ACS Symposium Series Applications in Forensic Proteomics: Protein Identification and Profiling. American Chemical Society. 2019; Vol. 1339; Chapter 10, 161-174. https://pubs.acs.org/doi/full/10.1021/bk-2019-1339.ch010
4. Schuit M, Gardner S, Wood S, Bower K, Williams G, Freeburger D, et al. The Influence of Simulated Sunlight on the Survival of Influenza virus in Aerosols. J Infect Dis. 2019; 221(3):372-378. https://www.ncbi.nlm.nih.gov/pubmed/31778532
5. Amarasinghe GK, Ayllón MA, Bào Y, Basler CF, Bavari S, Blasdell KR, et al. Taxonomy of the order Mononegavirales: update 2019. Arch Virol. 2019; 164(7):1967-1980. https://www.ncbi.nlm.nih.gov/pubmed/31089958
6. Schmidt C. Phage therapy's latest makeover. Nat Biotechnol. 2019; 37(6):581–586. https://www.nature.com/articles/s41587-019-0133-z

5. Briefly describe the biological defence work carried out at the facility, including type(s) of microorganisms* and/or toxins studied, as well as outdoor studies of biological aerosols:

***Objectives*:** The NBACC mission is to provide the nation with the scientific basis for characterization of biological threats and bioforensic analysis to support attribution investigations. NBACC conducts studies to fill in information gaps to better understand current and future biological threats; to assess vulnerabilities; and to determine potential impacts to guide the development of biological countermeasures such as detectors, drugs, vaccines, and decontamination technologies. When needed, NBACC conducts experimental programs to better characterize the benefits and risks of changes in U.S. biodefense preparations. NBACC also develops bioforensic assays and provides operational bioforensic analysis to support the attribution of biocrime and bioterrorism.

* Including viruses and prions.

***Microorganisms and/or Toxins Studied*:** Select Agents (HHS, Overlap), Select Toxins (HHS), simulants, NIAID Category A pathogens.

***Outdoor Studies*:** No outdoor studies performed.

Form A, Part 2 (iii)

National biological defence research and development programmes: Facilities

1. What is the name of the facility?
Plum Island Animal Disease Center (PIADC)

2. Where is it located (provide both address and geographical location)?
40550 Route 25, Orient Point, New York 11957

3. Floor area of laboratory areas by containment level (m^2):

BSL-2:	292 m^2
BSL-3:	18,046 m^2
BSL-4:	0 m^2
Total laboratory floor area:	18,338 m^2

4. The organizational structure of each facility:

(i)	**Total number of personnel:**	461
(ii)	**Division of personnel:**	
	Military	0
	Civilian	461
(iii)	**Division of personnel by category:**	
	Scientists	88
	Engineers	4
	Technicians	18
	Administrative and support staff	351

Note: The personnel increase from 367 in 2018 to 461 in 2019 is due to the opening of a new Waste Water Treatment Facility and re-staffing following renovation efforts that ended in 2019.

(b)(5)

(iv) List the scientific disciplines represented in the scientific/engineering staff:
Biological Science, Chemistry, Engineering, Microbiology, Molecular Biology, Computational Biology, Pathology, Veterinary Medicine

(v) Are contractor staff working in the facility? If so, provide an approximate number:
Yes Number: 328

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
U.S. Department of Agriculture (USDA)
U.S. Department of Homeland Security (DHS)

(vii) What are the funding levels for the following program areas:

Research	$ 7,839,954
Development	$ 1,800,000
Test and evaluation	$ 5,146,590
Total	$ 14,786,544

(viii) Briefly describe the publication policy of the facility:

DHS scientific research staffs are expected to publish papers in open literature. Papers are peer reviewed and approved by PIADC and DHS for security, clarity, and accuracy with regard to the description of work prior to submittal to journals or release. All USDA Agricultural Research Service (ARS) scientists are obligated to publish scientific research data in peer-reviewed publications after review for dual use determination (not all publications by these scientists are relevant to this report). ARS scientists are encouraged to present research at scientific conferences and to publish in books and proceedings. ARS maintains a searchable online database of publications by scientists (available at https://www.ars.usda.gov/research/publications/publications-at-this-location/?modeCode=80-64-05-00). USDA Animal and Plant Health Inspection Service diagnostic staff are encouraged to publish papers in journals or other formats that are available to the public. Papers follow the review process outlined in standard operating procedure (document number SOP-NVSL-0004) titled "Approval of Manuscripts and Abstracts for Publication, and Posters and Presentations for Display."

(ix) Provide a list of publicly-available papers and reports resulting from the work during the previous 12 months. (To include authors, titles, and full references.):

1. Arzt J, Bertram MR, Vu LT, Pauszek SJ, Hartwig EJ, Smoliga GR, et al. First Detection and Genome Sequence of Senecavirus A in Vietnam. Microbiol Resour Announc. 2019; 8(3):e01247-18. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6346150/
2. Arzt J, Fish I, Pauszek SJ, Johnson SL, Chain PS, Rai DK, et al. The evolution of a super-swarm of foot-and-mouth disease virus in cattle. PLoS ONE. 2019; 14(4):e0210847. https://doi.org/10.1371/journal.pone.0210847
3. Arzt J, Branan MA, Delgado AH, Yadav S, Moreno-Torres KI, Tildesley MJ, et al. Quantitative impacts of incubation phase transmission of foot-and-mouth disease virus. Sci Rep. 2019; 9(1):2707. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6389902/
4. Bertram MR, Dickmu S, Palinski RM, Pauszek SJ, Hartwig EJ, Smoliga GR, et al. Genome Sequences of Four Foot-and-Mouth Disease Virus SAT 1 Topotype X Isolates from Cameroon. Microbiol Resour Announc. 2019; 8(49):e01243-19. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6895307/
5. Bertram MR, Palinski RM, Ranjan R, Biswal JK, Pauszek SJ, Hartwig EJ, et al. Genome sequences of 18 foot-and-mouth disease virus outbreak strains of serotype O sublineage IND2001D from India, 2013 to 2014. Microbiol Resour Announc. 2019; 8(33):e00776-19. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6696650/
6. Biswal JK, Ranjan R, Subramaniam S, Mohapatra JK, Patidar S, Sharma MK, et al. Genetic and antigenic variation of foot-and-mouth disease virus during persistent infection in naturally infected cattle and Asian buffalo in India. PLoS ONE. 2019; 14(6):e0214832. https://doi.org/10.1371/journal.pone.0214832
7. Borca MV, Holinka LG, Ramirez-Medina E, Risatti GR, Vuono EA, Berggren KA, et al. Identification of structural glycoprotein E2 domain critical to mediate replication of Classical Swine Fever Virus in SK6 cells. Virology. 2019; 526:38-44. https://www.sciencedirect.com/science/article/pii/S004268221830309X?via%3Dihub
8. Borca MV, Vuono EA, Ramirez-Medina E, Azzinaro P, Berggren KA, Singer M, et al. Structural Glycoprotein E2 of Classical Swine Fever Virus Interacts with Host Protein Dynactin Subunit 6 (DCTN6) during the Virus Infectious Cycle. J Virol. 2019; 94(1):e01642-19. https://jvi.asm.org/content/94/1/e01642-19
9. Das A, Xu L, Jia W. Development of conventional and real time PCR assays for rapid species authentication of mammalian cell lines commonly used in veterinary diagnostic laboratories. Res Vet Sci. 2019; 126:170-177. https://www.sciencedirect.com/science/article/abs/pii/S0034528819303807?via%3Dihub

10. Fernandez-Sainz I, Gavitt TD, Koster M, Ramirez-Medina E, Rodriguez YY, Wu P, et al. The VP1 G-H loop hypervariable epitope contributes to protective immunity against Foot and Mouth Disease Virus in swine. Vaccine. 2019; 37(26):3435-3442. https://doi.org/10.1016/j.vaccine.2019.05.019
11. Fish IH, Vierra D, Ehizibolo DO, Palinski R, Bertram MR, Pauszek SJ, et al. Near-full-length genome sequence of a foot-and-mouth disease virus of serotype southern African territories 2 isolated from Nigeria in 2014. Microbiol Resour Announc. 2019; 8(35):e00788-19. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6715872/
12. Laughlin RC, Madera R, Peres Y, Berquist BR, Wang L, Buist S, et al. Plant-made E2 glycoprotein single-dose vaccine protects pigs against classical swine fever. Plant Biotechnol J. 2019; 17(2):410-420. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6335066/
13. Martel E, Forzono E, Kurker R, Clark BA, Neilan JG, Puckette M. Effect of foot-and-mouth disease virus 3C protease B2 beta-Strand proline mutagenesis on expression and processing of the P1polypeptide using a plasmid expression vector. J Gen Virol. 2019; 100(3):446-456. https://www.microbiologyresearch.org/content/journal/jgv/10.1099/jgv.0.001204#tab2
14. Munsey A, Mwiine FN, Ochwo S, Velazquez-Salinas L, Ahmed Z, Maree F, et al. Spatial distribution and risk factors for foot and mouth disease virus in Uganda: Opportunities for strategic surveillance. Prev Vet Med. 2019; 171(1):104766. https://doi.org/10.1016/j.prevetmed.2019.104766
15. Mwiine FN, Velazquez-Salinas L, Ahmed Z, Ochwo S, Munsey A, Kenney M, et al. Serological and phylogenetic characterization of foot and mouth disease viruses from Uganda during cross-sectional surveillance study in cattle between 2014 and 2017. Transbound Emerg Dis. 2019; 66(5):2011-2024. https://onlinelibrary.wiley.com/doi/abs/10.1111/tbed.13249
16. O'Donnell VK, Grau FR, Mayr GA, Sturgill Samayoa TL, Dodd KA, Barrette RW. Rapid sequence-based characterization of African swine fever virus using the Oxford Nanopore MinION sequence sensing device and a companion analysis software tool. J Clin Microbiol. 2019; 58(1):e01104-19. https://jcm.asm.org/content/58/1/e01104-19.long
17. Omondi G, Alkhamis MA, Obanda V, Gakuya F, Sangula A, Pauszek S, et al. Phylogeographical and cross-species transmission dynamics of SAT1 and SAT2 foot-and-mouth disease virus in Eastern Africa. Mol Ecol. 2019; 28(11):2903-2916. https://doi.org/10.1111/mec.15125
18. Palinski RM, Sangula A, Gakuya F, Bertram MR, Pauszek SJ, Hartwig EJ, et al. Foot-and-mouth disease virus serotype a genome sequence from Kenya in 2016. Microbiol Resour Announc. 2019; 8(38):e00987-19. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6753279/
19. Palinski RM, Sangula A, Gakuya, Bertram MR, Pauszek SJ, Hartwig EJ, et al. Genome sequences of foot-and-mouth disease virus SAT1 and SAT2 strains from Kenya in 2014 to 2016. Microbiol Resour Announc. 2019; 8(36):e00809-19. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6728644/
20. Palinski RM, Bertram MR, Vu LT, Pauszek SJ, Hartwig EJ, Smoliga GR, et al. First genome sequence of foot-and-mouth disease virus serotype O sublineage IND2001E from southern Vietnam. Microbiol Resour Announc. 2019; 8(10):e01424-18. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6406109/
21. Palinski RM, Sangula A, Gakuya F, Bertram MR, Pauszek SJ, Hartwig EJ, et al. First report of near-complete genome sequences o foot-and-mouth disease virus serotype O strains from Kenya. Microbiol Resour Announc. 2019; 8(35):e00808-19. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6715873/
22. Ramirez-Medina E, Vuono E, O'Donnell V, Holinka LG, Silva E, Rai A, et al. Differential effect of the deletion of African Swine Fever virus virulence-associated genes in the induction of attenuation of the highly virulent Georgia strain. Viruses. 2019; 11(7):e599. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6669436/
23. Sitt T, Kenney M, Barrera J, Pandya M, Eckstrom K, Warner M, et al. Duration of protection and humoral immunity induced by an adenovirus-vectored subunit vaccine for foot-and-mouth disease (FMD) in Holstein steers. Vaccine. 2019; 37(42):6221-6231. https://doi.org/10.1016/j.vaccine.2019.08.017

24. Stenfeldt C, Pacheco JM, Singanallur NB, Vosloo W, Rodriguez LL, Arzt J. Virulence beneath the fleece; a tale of foot-and-mouth disease virus pathogenesis in sheep. PLoS ONE. 2019; 14(12):e0227061. https://doi.org/10.1371/journal.pone.0227061
25. Velazquez-Salinas L, Pauszek SJ, Barrera J, Clark BA, Borca MV, Verdugo-Rodriguez A, et al. Validation of a site-specific recombination cloning technique for the rapid development of a full-length cDNA clone of a virulent field strain of vesicular stomatitis New Jersey virus. J Virol Methods. 2019; 265:113-116. https://doi.org/10.1016/j.jviromet.2019.01.003
26. Velazquez-Salinas L, Pauszek SJ, Rodriguez LL. Complete genome sequence of a representative New Jersey vesiculovirus strain, NJ03CpB, from a region of endemicity in southern Mexico. Microbiol Resour Announc. 2019; 8(25):e00499-19. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6588370/
27. Velazquez-Salinas L, Verdugo-Rodriguez A, Rodriguez LL, Borca MV. The role of interleukin 6 during viral infections. Front Microbiol. 2019; 10:1057. https://www.frontiersin.org/articles/10.3389/fmicb.2019.01057/full
28. Visser LJ, Medina GN, Rabouw HH, de Groot RJ, Langereis MA, de Los Santos T, et al. Foot-and-Mouth Disease Virus Leader Protease Cleaves G3BP1 and G3BP2 and Inhibits Stress Granule Formation. J Virol. 2019; 93(2):e00922-18. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6321903/
29. Vuono EA, Ramirez-Medina E, Holinka LG, Baker-Branstetter R, Borca MV, Gladue DP. Interaction of structural glycoprotein E2 of classical swine fever virus with protein phosphatase 1 catalytic subunit beta (PPP1CB). Viruses. 2019; 11(4):E307. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6521620/
30. Wang Y, Das A, Zheng W, Porter E, Xu L, Noll L, et al. Development and evaluation of multiplex real-time RT-PCR assays for the detection and differentiation of foot-and-mouth disease virus and Seneca Valley virus 1. Transbound Emerg Dis. 2019; 00:1-13. doi:10.1111/tbed.13373. https://onlinelibrary.wiley.com/doi/abs/10.1111/tbed.13373
31. Yadav S, Stenfeldt C, Branan MA, Moreno-Torres KI, Holmstrom LK, Delgado AH, et al. Parameterization of the Durations of Phases of Foot-And-Mouth Disease in Cattle. Front Vet Sci. 2019; 6:263. https://www.frontiersin.org/articles/10.3389/fvets.2019.00263/full
32. Zhu JJ, Canter JA, Rodriguez LL, Arzt J. A novel bovine CXCL15 gene in the GRO chemokine gene cluster. Vet Immunol Immunopathol. 2019; 220:109990. https://doi.org/10.1016/j.vetimm.2019.109990
33. Zhu JJ, Ramanathan P, Bishop EA, O'Donnell V, Gladue DP, Borca MV. Mechanisms of African swine fever virus pathogenesis and immune evasion inferred from gene expression changes in infected swine macrophages. PLoS One. 2019; 14(11):e0223955. https://doi.org/10.1371/journal.pone.0223955

5. Briefly describe the biological defence work carried out at the facility, including type(s) of microorganisms* and/or toxins studied, as well as outdoor studies of biological aerosols:

Objectives: PIADC provides the only research and development and confirmatory diagnostic capability for specific high-consequence, contagious, foreign animal diseases of livestock, including foot-and-mouth disease in the United States. Technologies researched and developed are vaccines, antivirals, and diagnostic methods.

Microorganisms and/or Toxins Studied: Select Agents (USDA)

Outdoor Studies: No outdoor studies performed

* Including viruses and prions.

Form A, Part 2 (iii)

National biological defence research and development programmes: Facilities

1. What is the name of the facility?
Lothar Salomon Test Facility (LSTF)

2. Where is it located (provide both address and geographical location)?
2029 Burns Road, TEDT-DPW-LS MS#6, Dugway, Utah 84022-5006

3. Floor area of laboratory areas by containment level (m^2):

BSL-2:	1,111 m^2
BSL-3:	1,174 m^2
BSL-4:	0 m^2
Total laboratory floor area:	2,285 m^2

Note: The increases in laboratory floor area are due to the inclusion of a recently constructed annex that was added to the existing lab structure.

(b)(5)

4. The organizational structure of each facility:

(i) Total number of personnel: 37

(ii) Division of personnel:

Military	0
Civilian	37

(iii) Division of personnel by category:

Scientists	20
Engineers	0
Technicians	8
Administrative and support staff	9

(iv) List the scientific disciplines represented in the scientific/engineering staff:
Aerobiology, Bacteriology, Biochemistry, Immunology, Microbiology, Molecular Biology, Toxicology, Virology

(v) Are contractor staff working in the facility? If so, provide an approximate number:
Yes. Number: 9

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
U.S. Department of Defeuse (DoD) – Wholly

(vii) What are the funding levels for the following program areas:

Research	$ 0
Development	$ 0
Test and evaluation	$ 843,008
Total	$ 843,008

(viii) Briefly describe the publication policy of the facility:
It is Army policy to encourage scientific and technical personnel to publish research procedures and results in recognized professional journals as well as present their work at national and international professional meetings. Such publication is an important part of the Army's research and development program.

Publications are prepared and published in accordance with Army regulations. The regulations governing the publication of research findings include:
AR 70-31 "Standards for Technical Reporting"
https://armypubs.army.mil/epubs/DR_pubs/DR_a/pdf/web/ARN4633_AR70-31_WEB_Final.pdf
AR 360-1 "The Army Public Affairs Program"
https://armypubs.army.mil/epubs/DR_pubs/DR_a/pdf/web/ARN6644_AR360-1_Admin_WEB_FINAL.pdf
AR 530-1 "Operations Security"
https://armypubs.army.mil/epubs/DR_pubs/DR_a/pdf/web/r530_1.pdf

Professional scientists are encouraged to publish papers in peer reviewed journals. All publications must obtain the necessary command and public affairs permission before submission. Release of DoD publications is guided by DoD Directive 5230.09, Clearance of DoD Information for Public Release (https://www.esd.whs.mil/Portals/54/Documents/DD/issuances/dodi/523029p.pdf pdf) and DoD Instruction 5320.29, Security and Policy Review of DoD Information for Public Release (http://www.esd.whs.mil/Portals/54/Documents/DD/issuances/dodi/523029p.pdf).

(ix) Provide a list of publicly-available papers and reports resulting from the work during the previous 12 months. (To include authors, titles, and full references.):

None

5. Briefly describe the biological defence work carried out at the facility, including type(s) of microorganisms* and/or toxins studied, as well as outdoor studies of biological aerosols:

Objectives: Testing battlefield detection and identification methods, protective equipment, and decontamination systems, including interferent testing of biological detectors and to develop/validate aerosol particle dispersion models to enhance countermeasure response.
https://www.dugway.army.mil/LifeSciences.aspx.

Microorganisms and/or Toxins Studied: None

Outdoor Studies: None

* Including viruses and prions.

Form A, Part 2 (iii)

National biological defence research and development programmes: Facilities

1. What is the name of the facility?
Naval Medical Research Center (NMRC)

2. Where is it located (provide both address and geographical location)?
8400 Research Plaza, Fort Detrick, Maryland 21702

3. Floor area of laboratory areas by containment level (m^2):

BSL-2:	2,000 m^2
BSL-3:	0 m^2
BSL-4:	0 m^2
Total laboratory floor area:	2,000 m^2

4. The organizational structure of each facility:

(i) Total number of personnel: 87

(ii) Division of personnel:

Military	19
Civilian	68

(iii) Division of personnel by category:

Scientists	14
Engineers	0
Technicians	65
Administrative and support staff	8

(iv) List the scientific disciplines represented in the scientific/engineering staff:
Biochemistry, Computational Biology, Immunology, Microbiology, Molecular Biology

(v) Are contractor staff working in the facility? If so, provide an approximate number:
Yes Number: 57

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
U.S. Department of Defense – Wholly

(vii) What are the funding levels for the following program areas:

Research	$ 20,704,000
Development	$ 0
Test and evaluation	$ 0
Total	$ 20,704,000

(viii) Briefly describe the publication policy of the facility:
Professional scientists are encouraged to publish worthy papers in peer reviewed journals. All publications must obtain the necessary command and public affairs clearance before submission. Release of DoD publications is guided by DoD Directive 5230.09, Clearance of DoD Information for Public

Release (https://www.esd.whs.mil/Portals/54/Documents/DD/issuances/dodi/523009p_1.pdf?ver=2019-06-26-120334-963) and DoD Instruction 5320.29, Security and Policy Review of DoD Information for Public Release (https://www.esd.whs.mil/Portals/54/Documents/DD/issuances/dodi/523029p.pdf)

(ix) Provide a list of publicly-available papers and reports resulting from the work during the previous 12 months. (To include authors, titles, and full references.):

1. Voegtly LJ, Rice GK, Cer RZ, Frey KG, Biswas B, Hamilton T, Aslam S, Bishop-Lilly KA. High quality draft genome sequence of Pseudomonas aeruginosa 268 isolated from a patient with left ventricular assist device. Microbiol Resource Announc. 2019 Jan 3;8(1). https://www.ncbi.nlm.nih.gov/pubmed/30637391
2. Millar EV, Rice GK, Schlett CD, Elassal EM, Cer RZ, Frey KG, Hamilton TC, Ellis MW, Tribble DR, Bishop-Lilly KA, Bennett JW. Genomic epidemiology of MRSA infection and colonization isolates among military trainees with skin and soft tissue infection. Infection. 2019 Oct;47(5):729-737. Epub 2019 Feb 22. https://www.ncbi.nlm.nih.gov/pubmed/30796628
3. Paskey AC, Frey KG, Schroth G, Gross S, Hamilton T, Bishop-Lilly KA. Enrichment Post-Library Preparation Enhances the Sensitivity of High-Throughput Sequencing-Based Detection and Characterization of Viruses from Complex Samples. BMC Genomics. 2019 Feb 26;20(1):155. doi: 10.1186/s12864-019-5543-2. https://www.ncbi.nlm.nih.gov/pubmed/30808306
4. Schully KL, Bell MG, Spall A, Yu K, Burtnick M, Mayo M, Theobald V, Clark DV, Maves RC, Currie BJ, Brett PJ, Lawler JV. Serological evidence of Burkholderia pseudomallei infection in US Marines who trained in Australia from 2012-2014: a retrospective analysis of archived specimens. MSMR. 2019 Jul; 26(7):8-17. https://www.ncbi.nlm.nih.gov/pubmed/31347371
5. Aslam S, Courtwright AM, Koval C, Lehman SM, Morales S, Furr CL, Rosas F, Brownstein MJ, Fackler JR, Sisson BM, Biswas B, Henry M, Luu T, Bivens BN, Hamilton T, Duplessis C, Logan C, Law N, Yung G, Turowski J, Ancsi J, Strathdee SA, Schooley RT. Early clinical experience of bacteriophage therapy in 3 lung transplant recipients. Am J Transplant. 2019 Sep; 19(9):2631-2639. Epub 2019 Jul 17. https://www.ncbi.nlm.nih.gov/pubmed/31207123
6. Krishnan S, Beckett C, Espinosa B, Clark DV. Austere environments Consortium for Enhanced Sepsis Outcomes (ACESO). Shock. 2019 Sep 17. Epub ahead of print. https://www.ncbi.nlm.nih.gov/pubmed/31568224
7. Berry IM, Melendrez MC, Bishop-Lilly KA, Rutvisuttinunt W, Pollett S, Talundzic E, Morton L, Jarman RG. Next generation sequencing and bioinformatics methodologies for infectious disease research and Public Health: Approaches, applications, and considerations for development of laboratory capacity. J Infect Dis. 2019 Oct 14. Epub ahead of print. https://www.ncbi.nlm.nih.gov/pubmed/31612214
8. Ko E, Philipson C, Burke TW, Cer RZ, Bishop-Lilly KA, Voegtly L, Tsalik EL, Woods CW, Clark D, Schully KL. Direct-from-blood RNA sequencing identifies the cause of fatal post-bronchoscopy fever. BMC Infect Dis. 2019 Oct 28; 19(1):905. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6819639/
9. Ecale Zhou CL, Maltatti S, Kimbrel J, Philipson C, McNair K, Hamilton T, Edwards R, Souza B. multiPhATE: bioinformatics pipeline for functional annotation of phage isolates. Bioinformatics. 2019 Nov 1;35(21):4402-4404. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6821344/
10. Nir-Paz R, Gelman D, Khouri A, Sisson B, Fackler J, Alkalai-Oren S, Khalifa L, Rimon A, Yeruchalmy O, Bader R, Amit S, Coppenhagen-Glazer S, Henry M, Quinones J, Malagon F, Biswas B, Moses AI, Merril G, Schooley RT, Brownstein MJ, Weil YA, Hazan R. Successful treatment of antibiotic resistant poly-microbial bone infection with bacteriophages. Clin Infect Dis. 2019 Nov 13;69(11):2015-2018. https://www.ncbi.nlm.nih.gov/pubmed/30869755

5. Briefly describe the biological defence work carried out at the facility, including type(s) of microorganisms* and/or toxins studied, as well as outdoor studies of biological aerosols:

Objectives: The goal of the program is the development of rapid and deployable detection assays to protect deployed troops. During 2019, we continued studying clinical cases of sepsis in austere environments with the ultimate goal of understanding host-pathogen interactions, development of new diagnostic assays and better treatment strategies against relevant infectious diseases. Additional efforts include: development of a diagnostic for Bacillus anthracis using phage combined with other technology, a program to improve over traditional diagnostics for Rickettsia prowazekii, and a program aimed at developing and testing a virus enrichment sequencing assay for viruses of biosurveillance and biodefense concern. Additional information is available at https://www.med.navy.mil/sites/nmrc/NMRC/Pages/NMRC.aspx

Microorganisms and/or Toxins Studied: Select Agents (HHS, Overlap) and HHS Select Toxins, NIAID Category A pathogens

Outdoor Studies: None

* Including viruses and prions.

Form A, Part 2 (iii)

National biological defence research and development programmes: Facilities

1. What is the name of the facility?
Naval Research Laboratory (NRL)

2. Where is it located (provide both address and geographical location)?
4555 Overlook Ave., SW, Washington, D.C. 20375

3. Floor area of laboratory areas by containment level (m^2):

BSL-1:	358 m^2
BSL-2:	394 m^2
BSL-3:	0 m^2
BSL-4:	0 m^2
Total laboratory floor area:	752 m^2

Note: The changes in NRL laboratory space from 2018 are due to shifting research and development activities. The laboratory areas were not physically remodeled.

(b)(5)

4. The organizational structure of each facility:

(i) Total number of personnel: 31

(ii) Division of personnel:

Military	1
Civilian	30

(iii) Division of personnel by category:

Scientists	25
Engineers	2
Technicians	4
Administrative and support staff	0

(iv) List the scientific disciplines represented in the scientific/engineering staff:
Biochemistry, Biophysics, Chemical Engineering, Chemistry, Electrical Engineering, Engineering, Immunology, Mechanical Engineering, Microbiology, Molecular Biology, Physics

(v) Are contractor staff working in the facility? If so, provide an approximate number:
Yes Number: 5

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
U.S. Department of Defense – Wholly

(vii) What are the funding levels for the following program areas:

Research	$ 2,400,000
Development	$ 3,800,000
Test and evaluation	$ 500,000
Total	$ 6,700,000

(viii) Briefly describe the publication policy of the facility:

Professional scientists are encouraged to publish papers in peer reviewed journals. All publications must obtain the necessary command and public affairs permission before submission. Release of DoD publications is guided by DoD Directive 5230.09 (Clearance of DoD Information for Public Release, https://www.esd.whs.mil/Portals/54/Documents/DD/issuances/dodi/523029p.pdf) and DoD Instruction 5320.29 (Security and Policy Review of DoD Information for Public Release, https://www.esd.whs.mil/Portals/54/Documents/DD/issuances/dodi/523009p_1.pdf?ver=2019-06-26-120334-963) for publishing information related to biological defense efforts. Public release of unclassified technical information is subject to sponsor approval.

(ix) Provide a list of publicly-available papers and reports resulting from the work during the previous 12 months. (To include authors, titles, and full references.):

1. Anderson, G.P., Liu, J.L., Shriver-Lake, L.C., Zabetakis, D., Sugiharto, V.A., Chen, H.-W., Lee, C.-R., Defang, G.N., Wu, S.-J.L., Venkateswaran, N., Goldman, E.R. Oriented Immobilization of Single-Domain Antibodies Using SpyTag/SpyCatcher Yields Improved Limits of Detection. Analytical Chemistry. 2019; 91 (15), pp. 9424-9429. doi: 10.1021/acs.analchem.9b02096. https://pubs.acs.org/doi/10.1021/acs.analchem.9b02096
2. Hu X, Compton, J.R., Legler, P.M. 2019. Analysis of Group IV viral SSHHPS Using In vitro and In silico Methods. JOVE In Press. 2019; https://www.ncbi.nlm.nih.gov/pubmed/31904018
3. Liu, J.L., Shriver-Lake, L.C., Zabetakis, D., Anderson, G.P., Goldman, E.R. Selection and characterization of protective anti-chikungunya virus single domain antibodies Molecular Immunology. 2019; 105, pp. 190-197. doi: 10.1016/j.molimm.2018.11.016 https://www.sciencedirect.com/science/article/pii/S0161589018306710?via%3Dihub
4. Lu, Q.; E. Barlow, D. E.; Haridas, D.; Giordano, B. C.; Ladouceur, H. D.; Gaston, J. D; Collins, G. E.; Terray, A. V. Flow-Through Optical Chromatography in Combination with Confocal Raman Microspectroscopy: A Novel Label-Free Approach To Detect Responses of Live Macrophages to Environmental Stimuli. ACS Omega. 2019; 4, 12938−12947 (18-1231-3702) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6682052/
5. Morazzani EM, Compton JR, Leary DH, Berry AV, Hu X, Marugan J, Glass PJ, Legler PM. Proteolytic cleavage of host proteins by the Group IV viral proteases of Venezuelan equine encephalitis virus and Zika virus. Antiviral Research. 2019; 164:106-122. https://www.ncbi.nlm.nih.gov/pubmed/30742841
6. Olson MA, Legler PM, Zabetakis D, Turner KB, Anderson GP, Goldman ER. Sequence Tolerance of a Single-Domain Antibody with a High Thermal Stability: Comparison of Computational and Experimental Fitness Profiles. ACS Omega. 2019; 4:10444-10454. https://pubs.acs.org/doi/pdf/10.1021/acsomega.9b00730
7. Zabetakis, D., Shriver-Lake, L.C., Olson, M.A., Goldman, E.R., Anderson, G.P. Experimental evaluation of single-domain antibodies predicted by molecular dynamics simulations to have elevated thermal stability. Protein Science. 2019; 28:1909–1912. doi: 10.1002/pro.3692. https://onlinelibrary.wiley.com/doi/abs/10.1002/pro.3692

5. Briefly describe the biological defence work carried out at the facility, including type(s) of microorganisms* and/or toxins studied, as well as outdoor studies of biological aerosols:

* Including viruses and prions.

Objectives: The objectives of research at NRL are to develop and test reliable systems for the detection of chemical and biological (CB) warfare agents in order to provide early warning and contamination avoidance information. Additional information is available at http://www.nrl.navy.mil/research/

Microorganisms and/or Toxins Studied: Simulants of Select Agents (HHS, Overlap) and Toxins, NIAID Category A pathogens.

Outdoor Studies: None

Form A, Part 2 (iii)

National biological defence research and development programmes: Facilities

1. What is the name of the facility?
Naval Surface Warfare Center-Dahlgren Division, Chemical, Biological, Radiological (CBR) Defense Laboratory

2. Where is it located (provide both address and geographical location)?
6149 Welsh Road, Dahlgren, Virginia 22448

3. Floor area of laboratory areas by containment level (m^2):

BSL-2:	180 m^2
BSL-3:	27 m^2
BSL-4:	0 m^2
Total laboratory floor area:	207 m^2

4. The organizational structure of each facility:

(i) Total number of personnel: 76

(ii) Division of personnel:

Military	0
Civilian	76

(iii) Division of personnel by category:

Scientists	39
Engineers	17
Technicians	13
Administrative and support staff	7

(iv) List the scientific disciplines represented in the scientific/engineering staff:
Aerospace Engineering, Chemical Engineering, Chemistry, Computer Engineering, Computer Science, Electronic Engineering, Industrial Engineering, Mathematics, Mechanical Engineering, Microbiology, Molecular Biology, Operations Research Analysis, Physics, Toxicology

(v) Are contractor staff working in the facility? If so, provide an approximate number:
Yes Number: 2

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
U.S. Department of Defense (DoD) – Partly
Private Sector Companies
Internal (Laboratory Directed Research and Development [LDRD])
Other Governmental Agencies

(vii) What are the funding levels for the following program areas:

Research	$ 1,902,265
Development	$ 4,225,959
Test and evaluation	$ 159,794
Total	$ 6,288,018

(viii) Briefly describe the publication policy of the facility:
Employees are encouraged to publish. Employees must follow appropriate U.S. DoD guidelines for publishing information related to biological defense efforts and have all publications approved. Public release of unclassified technical information is subject to sponsor approval.

Release of DoD publications is guided by DoD Directive 5230.09, Clearance of DoD Information for Public Release (https://www.esd.whs.mil/Portals/54/Documents/DD/issuances/dodi/523029p.pdf) and DoD Instruction 5320.29, Security and Policy Review of DoD Information for Public Release (https://www.esd.whs.mil/Portals/54/Documents/DD/issuances/dodi/523009p_1.pdf?ver=2019-06-26-120334-963)

(ix) Provide a list of publicly-available papers and reports resulting from the work during the previous 12 months. (To include authors, titles, and full references.):

1. Standard Practice for Microbial Preservation. ASTM E35.15 Work Item 63006. 2019. https://www.astm.org/DATABASE.CART/WORKITEMS/WK63006.htm
2. Tony L. Buhr, Neil Kennihan, Zachary A. Minter, Alice Young, Erica Borgers-Klonkowski, Emily Osborn, Matt Bohmke, Shelia Hamilton, Monique Kimani, Andrea Staab, Mark Hammon, Charles Miller, Ryan Mackie, Sam Lilly. Combining Spore Germination and Heat Inactivation to Decontaminate Materials Contaminated with Bacillus anthracis Spores. Accepted by J Appl Microbiol. 2019; https://www.ncbi.nlm.nih.gov/pubmed/31573710
3. Cote, C., Buhr, T., Bernhards, C.B., Bohmke, M.D., Calm, A.M., Esteban-Trexler, J.S., Hunter, M., Katoski, S.E., Kennihan, N., Klimko, C.P., Miller, J.A., Minter, Z.A, Pfarr, J.W., Prugh, A.M., Quirk, A.V., Rivers, B.A., Shea, A.A., Shoe, J.L., Sickler, T.M., Young, A.A., Fetterer, D.P., Welkos, S.L., Bozue, J.A., McPherson, D., Fountain, A.W. III, and Gibbons, H.S. A Standard Method to Inactivate Bacillus anthracis Spores to Sterility via Gamma Irradiation. Appl Environ Microbiol. 2018. 84:e00106-18. https://www.ncbi.nlm.nih.gov/m/pubmed/29654186/

5. Briefly describe the biological defence work carried out at the facility, including type(s) of microorganisms* and/or toxins studied, as well as outdoor studies of biological aerosols:

Objectives: Efforts at this defense laboratory are focused on biological detection systems, collective and individual protection systems, hazard mitigation technologies, risk assessment tools and consequence management planning.

Microorganisms and/or Toxins Studied: Overlap Select Agent + NIAID Category A, and simulants

Outdoor Studies: Performance testing of a prototype biosurveillance system using a biological simulant.

Note: The NSWC Dahlgren Chemical and Biological Defense Division in large part was moved to NSWC Indian Head over the past year. The only remaining portion of the division at NSWC Dahlgren is the technical, laboratory-focused aspect. All of the collective protection programs and support programs were moved to Indian Head. Therefore, a significant number of programs, personnel and funding were terminated, or re-allocated during the year. This accounts for the decrease in personnel, specifically non-technical personnel, as well as the decrease in funding for the division.

(b)(5)

* Including viruses and prions.

Form A, Part 2 (iii)

National biological defence research and development programmes: Facilities

1. What is the name of the facility?
U.S. Army Combat Capabilities Development Command Chemical and Biological Center (CCDC CBC), formerly named U.S. Army Edgewood Chemical and Biological Center (ECBC).

2. Where is it located (provide both address and geographical location)?
5183 Blackhawk Road, Aberdeen Proving Ground, Maryland 21010-5424

3. Floor area of laboratory areas by containment level (m^2):

BSL-2:	403 m2
BSL-3:	177 m2
BSL-4:	0 m2
Total laboratory floor area:	580 m2

Note: The BSL-2 laboratory space used for biodefense research and development was decreased from 532 to 403 m^2. The BSL-2 laboratory space was not physically remodeled.

4. The organizational structure of each facility:

(i) Total number of personnel 75

(ii) Division of personnel:

Military	0
Civilian	75

(iii) Division of personnel by category:

Scientists	54
Engineers	3
Technicians	18
Administrative and support staff	0

(iv) List the scientific disciplines represented in the scientific/engineering staff.
Aerobiology, Aerospace Engineering, Biochemistry, Biomedical Engineering, Biotechnology, Chemical Engineering, Chemistry, Computer Engineering, Immunology, Mathematics, Mechanical Engineering, Microbiology, Molecular Biology, Operations Research Analysis, Physics, Physiology, Toxicology, Toxinology, Virology

(v) Are contractor staff working in the facility? If so, provide an approximate number.
Yes Number: 5

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
U. S. Department of Defense (DoD) – Wholly

(vii) What are the funding levels for the following programme areas:

Research	$15,797,000
Development	$ 6,108,000
Test and evaluation	$ 0

Total $21,905,000

(viii) Briefly describe the publication policy of the facility:
It is Army policy to encourage scientific and technical personnel to publish research procedures and results in recognized professional journals as well as present their work at national and international professional meetings. Such publication is an important part of the Army's research and development program.

Publications are prepared and published in accordance with Army regulations. The regulations governing the publication of research findings include:
AR 70-31 "Standards for Technical Reporting"
https://armypubs.army.mil/epubs/DR_pubs/DR_a/pdf/web/ARN4633_AR70-31_WEB_Final.pdf
AR 360-1 "The Army Public Affairs Program"
https://armypubs.army.mil/epubs/DR_pubs/DR_a/pdf/web/ARN6644_AR360-1_Admin_WEB_FINAL.pdf
AR 530-1 "Operations Security"
https://armypubs.army.mil/epubs/DR_pubs/DR_a/pdf/web/r530_1.pdf

Professional scientists are encouraged to publish papers in peer reviewed journals. All publications must obtain the necessary command and public affairs permission before submission. Release of DoD publications is guided by DoD Directive 5230.09, Clearance of DoD Information for Public Release (https://www.esd.whs.mil/Portals/54/Documents/DD/issuances/dodi/523029p.pdf) and DoD Instruction 5320.29, Security and Policy Review of DoD Information for Public Release (http://www.esd.whs.mil/Portals/54/Documents/DD/issuances/dodi/523029p.pdf).

(ix) Provide a list of publicly-available papers and reports resulting from the work published during the previous 12 months (include authors, titles and full references.)

1. Buckley PE, Calm AM, Welsh H, Thompson R, Menking D, Carney J, Warner C, Zacharko M. DARPA Antibody Technology Program, Phase II: Characterization of an Anti-BclA Antibody Produced by U.S. Naval Research Laboratory. Combat Capabilities Development Command – Chemical Biological Center. 2019, CCDC CBC-TR-1570. DOI: https://apps.dtic.mil/dtic/tr/fulltext/u2/1074346.pdf
2. Buckley PE, Calm AM, Welsh H, Thompson R, Menking D, Carney J, Warner C, Zacharko, M. DARPA Antibody Technology Program, Phase II: Characterization of an Anti-HA33A Human Immunoglobulin G Antibody Produced by AnaptysBio, Inc. Combat Capabilities Development Command – Chemical Biological Center. 2019, CCDC CBC-TR-1571. DOI: https://apps.dtic.mil/dtic/tr/fulltext/u2/1074350.pdf.
3. Chandrasekar R, Lapin ZJ, Nichols AS, Braun RM, Fountain AW. Photonic integrated circuits for Department of Defense-relevant chemical and biological sensing applications: state-of-the-art and future outlooks. Opt Eng. 2019;58(2): 020901. https://doi.org/10.1117/1.OE.58.2.020901
4. Cole SD, Beabout K, Turner KB, Smith ZK, Funk VL, Harbaugh SV, Liem AT, Roth PA, Geier BA, Emanuel PA, Walper SA, Chavez JL, Lux MW. Quantification of Interlaboratory Cell-Free Protein Synthesis Variability. ACS Synth Biol. 2019;8(9):2080-91. https://www.ncbi.nlm.nih.gov/pubmed/31386355
5. Ibrahim MS, Angelini D, Prugh A, Sickler T, Biggs T, Harris J, Ziemski M. Methods for Inactivation of Venezuelan Equine Encephalitis Virus. Combat Capabilities Development Command – Chemical Biological Center. 2019, CCDC CBC-TR-1579. DOI: https://apps.dtic.mil/dtic/tr/fulltext/u2/1074319.pdf
6. Jabbour RE, Emmons, E, Tripathi A, Kunkel K. Effect of Homoserine Lactones on the Physical Properties of Bacterial Nanocellulose Materials. Combat Capabilities Development Command –

Chemical Biological Center. 2019, CCDC CBC-TR-1566. DOI: https://apps.dtic.mil/dtic/tr/fulltext/u2/1076329.pdf
7. Kesavan J, Kilper G, Williamson M, Alstadt V, Dimmock A, Bascom R. Laboratory Validation and Initial Field Testing of an Unobtrusive Bioaerosol Detector for Health Care Settings. Aerosol Air Qual Res. 2019;19(2):331-44. https://pennstate.pure.elsevier.com/en/publications/laboratory-validation-and-initial-field-testing-of-an-unobtrusive
8. McBride EM, Mach PM, Dhummakupt ES, Dowling S, Carmany DO, Demond PS, Rizzo G, Manicke NE, Glaros T. Paper spray ionization: Applications and perspectives. Trends Analyt Chem. 2019;118:722-30. DOI: https://www.sciencedirect.com/science/article/pii/S0165993619301037
9. Mehta HH, Prater AG, Beabout K, Elworth RAL, Karavis M, Gibbons HS, Shamoo Y. The Essential Role of Hypermutation in Rapid Adaptation to Antibiotic Stress. Antimicrob Agents Chemother. 2019;63(7): e00744-19. DOI: https://aac.asm.org/content/63/7/e00744-19
10. Menking, DL, Fruchey, IR, Buckley, PA, Rhea, KA. Evaluation of the Octet Biolayer Interferometer Platform for Biological Agent Environmental Sampling. Combat Capabilities Development Command – Chemical Biological Center. 2019, CCDC CBC-TR-1585. DOI: https://apps.dtic.mil/dtic/tr/fulltext/u2/1077415.pdf
11. Pomerantz NL, Anderson EE, Dugan NP, Hoffman NF, Barton HF, Lee DT, Oldham CJ, Peterson GW, Parsons GN. Air, Water Vapor, and Aerosol Transport through Textiles with Surface Functional Coatings of Metal Oxides and Metal-Organic Frameworks. ACS Appl Mater Interfaces. 2019;11(27):24683-90. DOI: https://pubs.acs.org/doi/10.1021/acsami.9b04091
12. Wichert WRA, Dhummakupt ES, Zhang CS, Mach PM, Bernhards RC, Glaros T, Manicke NE. Detection of Protein Toxin Simulants from Contaminated Surfaces by Paper Spray Mass Spectrometry. J Am Soc Mass Spectrom. 2019;30(8):1406-15. DOI: https://link.springer.com/article/10.1007/s13361-019-02141-6

5. Briefly describe the biological defence work carried out at the facility, including type(s) of micro-organisms* and or toxins studied, as well as outdoor studies of biological aerosols.

Objectives: Development of non-medical defensive material against biological agents including: research, development, and engineering for methods of rapid detection, identification, decontamination, and physical protection from biological threat agents. Additional information is available at https://www.cbc.ecdc.army.mil/.

Microorganisms and/or Toxins Studied: Select Agents and Toxins (HHS and Overlap Select Agents, NIAID Category A pathogens, and HHS Select Toxins) and Simulants

Outdoor Studies: None

* Including viruses and prions.

Form A, Part 2 (iii)

National biological defence research and development programmes: Facilities

1. What is the name of the facility?
U.S. Army Medical Research Institute of Chemical Defense (USAMRICD)

2. Where is it located (provide both address and geographical location)?
2900 Ricketts Point Road, Aberdeen Proving Ground, Maryland 21010

3. Floor area of laboratory areas by containment level (m^2):

BSL-2:	315 m^2
BSL-3:	0 m^2
BSL-4:	0 m^2
Total laboratory floor area:	315 m^2

4. The organizational structure of each facility:

(i) Total number of personnel: 18

(ii) Division of personnel:

Military	1
Civilian	17

(iii) Division of personnel by category:

Scientists	3
Engineers	0
Technicians	15
Administrative and support staff	0

(iv) List the scientific disciplines represented in the scientific/engineering staff:
Biochemistry, Molecular Biology, Pharmacology, Physiology, Neurotoxicology, Neuroscience

(v) Are contractor staff working in the facility? If so, provide an approximate number:
Yes Number: 15

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
U.S. Department of Defense (DoD),
U.S. National Institutes of Health (NIH)

(vii) What are the funding levels for the following program areas:

Research	$ 650,000
Development	$ 0
Test and evaluation	$ 0
Total	$ 650,000

(viii) Briefly describe the publication policy of the facility:
It is Army policy to encourage scientific and technical personnel to publish research procedures and results in recognized professional journals as well as present their work at national and international

professional meetings. Such publication is an important part of the Army's research and development program.

Publications are prepared and published in accordance with Army regulations. The regulations governing the publication of research findings include:
AR 70-31 "Standards for Technical Reporting"
https://armypubs.army.mil/epubs/DR_pubs/DR_a/pdf/web/ARN4633_AR70-31_WEB_Final.pdfAR 360-1 "The Army Public Affairs Program"
https://armypubs.army.mil/epubs/DR_pubs/DR_a/pdf/web/ARN6644_AR360-1_Admin_WEB_FINAL.pdfAR 530-1 "Operations Security"
https://armypubs.army.mil/epubs/DR_pubs/DR_a/pdf/web/r530_1.pdf

Professional scientists are encouraged to publish papers in peer reviewed journals. All publications must obtain the necessary command and public affairs permission before submission. Release of DoD publications is guided by DoD Directive 5230.09, Clearance of DoD Information for Public Release (https://www.esd.whs.mil/Portals/54/Documents/DD/issuances/dodi/523029p.pdf) and DoD Instruction 5320.29, Security and Policy Review of DoD Information for Public Release (http://www.esd.whs.mil/Portals/54/Documents/DD/issuances/dodi/523029p.pdf).

(ix) Provide a list of publicly-available papers and reports resulting from the work during the previous 12 months. (To include authors, titles, and full references.):

None

5. Briefly describe the biological defence work carried out at the facility, including type(s) of microorganisms[*] and/or toxins studied, as well as outdoor studies of biological aerosols:

Objectives: Discover and develop medical products and knowledge solutions against toxin threats through research, education and training, and consultation. USAMRICD performs comprehensive, basic scientific research using established and emerging technologies that support the transition of products to advanced development; develops education and training capabilities for military, interagency, domestic, and international personnel in the medical management of chemical casualties; and provides a venue for mutually beneficial collaboration with external investigators and interagency partners to conduct medical chemical defense research against chemical warfare agents and toxins. See more at:
http://usamricd.apgea.army.mil/

***Microorganisms and/or Toxins Studied*:** HHS Select Toxin

Outdoor Studies: None

[*] Including viruses and prions.

Form A, Part 2(iii)

National biological defence research and development programmes: Facilities

1. What is the name of the facility?
U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID)

2. Where is it located (provide both address and geographical location)?
1425 Porter Street, Fort Detrick, Frederick, Maryland 21702

3. Floor area of laboratory areas by containment level (m^2):

BSL-2:	26,026 m^2
BSL-3:	3,139 m^2
BSL-4:	1,186 m^2
Total laboratory floor area:	30,351 m2

4. The organizational structure of each facility:

(i) Total number of personnel 746

(ii) Division of personnel:

Military	182
Civilian	564

(iii) Division of personnel by category:

Scientists	202
Engineers	5
Technicians	311
Administrative and support staff	228

(iv) List the scientific disciplines represented in the scientific/engineering staff.
Aerobiology, Biochemistry, Chemistry, Clinical Immunology, Entomology, Genetics, Immunology, Microbiology, Molecular Biology, Toxicology, Veterinary Medicine, Virology.

(v) Are contractor staff working in the facility? If so, provide an approximate number.
Yes Number: 339

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
U.S. Department of Defense (DoD) – Partly
U.S. Department of Homeland Security (DHS)
U.S. Department of Health and Human Services (DHHS)
U.S. Department of Agriculture (USDA)
U.S. Department of Energy (DoE)
Universities
Private sector companies

(vii) What are the funding levels for the following programme areas:

Research	$ 1,680,576.90
Development	$ 42,861,912.55*
Test and evaluation	$ 13,070,705.55

Total	$ 57,613,195.00

*Includes reimbursables from Cooperative Research and Development Agreements and other Departments, which cannot be differentiated by the above categories.

(viii) Briefly describe the publication policy of the facility:
It is Army policy to encourage scientific and technical personnel to publish research procedures and results in recognized professional journals as well as present their work at national and international professional meetings. Such publication is an important part of the Army's research and development program.

Publications are prepared and published in accordance with Army regulations. The regulations governing the publication of research findings include:
AR 70-31 "Standards for Technical Reporting"
https://armypubs.army.mil/epubs/DR_pubs/DR_a/pdf/web/ARN4633_AR70-31_WEB_Final.pdf
AR 360-1 "The Army Public Affairs Program"
https://armypubs.army.mil/epubs/DR_pubs/DR_a/pdf/web/ARN6644_AR360-1_Admin_WEB_FINAL.pdf
AR 530-1 "Operations Security"
https://armypubs.army.mil/epubs/DR_pubs/DR_a/pdf/web/r530_1.pdf
Professional scientists are encouraged to publish papers in peer reviewed journals. All publications must obtain the necessary command and public affairs permission before submission. Release of DoD publications is guided by DoD Directive 5230.09, Clearance of DoD Information for Public Release (https://www.esd.whs.mil/Portals/54/Documents/DD/issuances/dodi/523029p.pdf) and DoD Instruction 5320.29, Security and Policy Review of DoD Information for Public Release (http://www.esd.whs.mil/Portals/54/Documents/DD/issuances/dodi/523029p.pdf).

(ix) Provide a list of publicly-available papers and reports resulting from the work published during the previous 12 months (include authors, titles and full references.)

1. Abudurexiti, A, Adkins, S, Alioto, D, Alkhovsky, SV, Avšič-Županc, et al. Taxonomy of the order Bunyavirales: update 2019. *Arch Virol.* 2019;164(7):1949-1965. DOI: https://link.springer.com/article/10.1007%2Fs00705-019-04253-6
2. Akhvlediani, T, Chitadze, N, Chlikadze, R, Rostiashvili, N, Betashvili, M, et al. Multivariate relationships between epidemiologic risk factors and zoonotic infections among military personnel in the country of Georgia: A non-linear canonical correlation analysis. *Zoonoses Public Health.* 2019;66(7):835-841. DOI: https://www.ncbi.nlm.nih.gov/pubmed/31338987
3. Amarasinghe, GK, Ayllón, MA, Bào, Y, Basler, CF, Bavari, S, et al. Taxonomy of the order Mononegavirales: update 2019. *Arch Virol.* 2019;164(7):1967-1980. DOI: https://link.springer.com/article/10.1007/s00705-019-04247-4
4. Amemiya, K, Dankmeyer, JL, Biryukov, SS, Trevino, SR, Kliuko, CP, et al. Deletion of two genes in Burkholderia pseudomallei MSHR668 that target essential amino acids protect acutely infected BALB/c mice and promote long term survival. *Vaccines (Basel).* 2019;7(4). DOI: https://www.mdpi.com/2076-393X/7/4/196
5. Atre, T, Phillips, RL, Modjarrad, K, Regules, JA, Bergmann-Leitner, ES. Development and characterization of a Zaire Ebola (ZEBOV) specific IgM ELISA. *J Immunol Methods.* 2019;468:29-34. DOI: https://www.ncbi.nlm.nih.gov/pubmed/30910536
6. Bachert, BA, Biryukov, SS, Chua, J, Rodriguez, SA, Toothman, RG, Jr., et al. A Francisella novicida mutant, lacking the soluble lytic transglycosylase Slt, exhibits defects in both growth and virulence.

Front Microbiol. 2019;10:1343. DOI: https://www.frontiersin.org/articles/10.3389/fmicb.2019.01343/full
7. Barnes, KB, Zumbrun, SD, Halasohoris, SA, Desai, PD, Miller, LL, et al. Demonstration of the broad spectrum in vitro activity of finafloxacin against pathogens of biodefence interest. *Antimicrob Agents Chemother.* 2019. DOI: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6879258/
8. Bazzill, JD, Stronsky, SM, Kalinyak, LC, Ochyl, LJ, Steffens, JT, et al. Vaccine nanoparticles displaying recombinant Ebola virus glycoprotein for induction of potent antibody and polyfunctional T cell responses. *Nanomedicine.* 2019;18:414-425. DOI: https://www.sciencedirect.com/science/article/abs/pii/S1549963418305562?via%3Dihub
9. Beitzel, B, Hulseberg, CE, Palacios, G. Reverse genetics systems as tools to overcome the genetic diversity of Lassa virus. *Curr Opin Virol.* 2019;37:91-96. DOI: https://www.sciencedirect.com/science/article/abs/pii/S1879625719300379?via%3Dihub
10. Blair, PW, Kuhn, JH, Pecor, DB, Apanaskevich, DA, Kortepeter, MG, et al. An emerging biothreat: Crimean-Congo hemorrhagic fever virus in Southern and Western Asia. *Am J Trop Med Hyg.* 2019;100(1):16-23. DOI: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6335890/
11. Bocan, TM, Basuli, F, Stafford, RG, Brown, JL, Zhang, X, et al. Synthesis of [18 F]Favipiravir and Biodistribution in C3H/HeN mice as assessed by Positron Emission Tomography. *Sci Rep.* 2019;9(1). DOI: https://www.nature.com/articles/s41598-018-37866-z
12. Bocan, TM, Stafford, RG, Brown, JL, Akuoku Frimpong, J, Basuli, F, , et al. Characterization of brain inflammation, apoptosis, hypoxia, blood-brain barrier integrity and metabolism in Venezuelan equine encephalitis virus (VEEV TC-83) exposed mice by in vivo positron emission tomography imaging. *Viruses.* 2019;11(11). DOI: https://www.mdpi.com/1999-4915/11/11/1052
13. Bornholdt, ZA, Herbert, AS, Mire, CE, He, S, Cross, RW, et al. A two-antibody pan-Ebolavirus cocktail confers broad therapeutic protection in ferrets and nonhuman primates. *Cell Host Microbe.* 2019;25(1):49-58.e45. DOI: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6341996/
14. Bose, ME, Shrivastava, S, He, J, Nelson, MI, Bera, J, et al. Sequencing and analysis of globally obtained human parainfluenza viruses 1 and 3 genomes. *PLoS ONE.* 2019;14(7). DOI: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0220057
15. Bournazos, S, DiLillo, DJ, Goff, AJ, Glass, PJ, Ravetch, JV. Differential requirements for FcγR engagement by protective antibodies against Ebola virus. *Proc Natl Acad Sci U S A.* 2019;116(40):20054-20062. DOI: https://www.pnas.org/content/116/40/20054.long
16. Bower, WA, Schiffer, J, Atmar, RL, Keitel, WA, Friedlander, AM, et al. Use of Anthrax Vaccine in the United States: recommendations of the Advisory Committee on Immunization Practices, 2019. *MMWR Recomm Rep.* 2019;68(4):1-14. DOI: https://www.cdc.gov/mmwr/volumes/68/rr/rr6804a1.htm
17. Brannan, JM, He, S, Howell, KA, Prugar, LI, Zhu, W, et al. Post-exposure immunotherapy for two ebolaviruses and Marburg virus in nonhuman primates. *Nat Commun.* 2019;10(1):105. DOI: https://www.nature.com/articles/s41467-018-08040-w
18. Brocato, RL, Hooper, JW. Progress on the prevention and treatment of hantavirus disease. *Viruses.* 2019;11(7):E610. DOI: https://www.mdpi.com/1999-4915/11/7/610
19. Burke, CW, Froude, JW, Rossi, F, White, CE, Moyer, CL, et al. Therapeutic monoclonal antibody treatment protects nonhuman primates from severe Venezuelan equine encephalitis virus disease after aerosol exposure. *PLoS Pathog.* 2019;15(12):e1008157. DOI: https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1008157
20. Cai, Y, Yu, S, Jangra, RK, Postnikova, EN, Wada, J, et al. Human, nonhuman primate, and bat cells are broadly susceptible to tibrovirus particle cell entry. *Front Microbiol.* 2019;10(APR). DOI: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6499107/
21. Calisher, CH, Briese, T, Brister, JR, Charrel, RN, Durrwald, R, et al. Strengthening the interaction of the virology community with the International Committee on Taxonomy of Viruses (ICTV) by

linking virus names and their abbreviations to virus species. *Syst Biol.* 2019;68(5):828-839. DOI: https://academic.oup.com/sysbio/article/68/5/828/5267841

22. Choi, JY, Hii, KC, Bailey, ES, Chuang, JY, Tang, WY, et al. Burkholderia pseudomallei detection among hospitalized patients, Sarawak. *Am J Trop Med Hyg.* 2019. DOI: http://www.ajtmh.org/content/journals/10.4269/ajtmh.19-0625
23. Chua, J, Bozue, JA, Klimko, CP, Shoe, JL, Ruiz, SI, et al. Formaldehyde and glutaraldehyde inactivation of bacterial tier 1 select agents in tissues. *Emerg Infect Dis.* 2019;25(5):919-926. DOI: https://wwwnc.cdc.gov/eid/article/25/5/18-0928_article
24. Clements, TL, Rossi, CA, Irish, AK, Kibuuka, H, Eller, LA, et al. Chikungunya and o'nyong-nyong viruses in Uganda: implications for diagnostics. *Open Forum Infect Dis.* 2019;6(3). DOI: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6411207/
25. Cooper, TK, Huzella, L, Johnson, JC, Rojas, O, Yellayi, S, , et al. Author Correction: Histology, immunohistochemistry, and in situ hybridization reveal overlooked Ebola virus target tissues in the Ebola virus disease guinea pig model. *Sci Rep.* 2019;9(1):15013. DOI: https://www.nature.com/articles/s41598-019-51386-4
26. Culbreth, MJ, Biryukov, SS, Shoe, JL, Dankmeyer, JL, Hunter, M, et al. The Use of analgesics during vaccination with a live attenuated Yersinia pestis vaccine alters the resulting immune response in mice. *Vaccines (Basel).* 2019;7(4):E205. DOI: https://www.mdpi.com/2076-393X/7/4/205
27. Davis, AZ, Jaffe, DA, Honadel, TE, Lapsley, WD, Wilber-Raymond, JL, et al. Prevalence of Bartonella sp. in United States military working dogs with infectious endocarditis: a retrospective case-control study. *J Vet Cardiol.* 2019;27:1-9. DOI: https://www.sciencedirect.com/science/article/abs/pii/S1760273419301468?via%3Dihub
28. DeShazer, D. A novel contact-independent T6SS that maintains redox homeostasis via Zn2+ and Mn2+ acquisition is conserved in the Burkholderia pseudomallei complex. *Microbiol Res.* 2019;226:48-54. DOI: https://www.sciencedirect.com/science/article/pii/S0944501319303192?via%3Dihub
29. DeShazer, D, Lovett, S, Richardson, J, Koroleva, G, Kuehl, K, et al. Bacteriophage-associated genes responsible for the widely divergent phenotypes of variants of Burkholderia pseudomallei strain MSHR5848. *J Med Microbiol.* 2019;68(2):263-278. DOI: https://www.microbiologyresearch.org/content/journal/jmm/10.1099/jmm.0.000908
30. Diagne, MM, Ndione, MHD, Di Paola, N, Fall, G, Bedekelabou, AP, et al. Usutu virus isolated from rodents in Senegal. *Viruses.* 2019;11(2):E181. DOI: https://www.mdpi.com/1999-4915/11/2/181
31. Espy, N, Nagle, E, Pfeffer, B, Garcia, K, Chitty, AJ, et al. T-705 induces lethal mutagenesis in Ebola and Marburg populations in macaques. *Antiviral Res.* 2019;170:104529. DOI: https://www.sciencedirect.com/science/article/abs/pii/S0166354219303109?via%3Dihub
32. Fan, Y, Stronsky, SM, Xu, Y, Steffens, JT, van Tongeren, SA, et al. Multilamellar vaccine particle elicits potent immune activation with protein antigens and protects mice against Ebola virus infection. *ACS Nano.* 2019;13(10): 11087-11096. DOI: https://pubs.acs.org/doi/10.1021/acsnano.9b03660
33. Fathy Mohamed, Y, Scott, NE, Molinaro, A, Creuzenet, C, Ortega, X, et al. A general protein O-glycosylation machinery conserved in Burkholderia species improves bacterial fitness and elicits glycan immunogenicity in humans. *J Biol Chem.* 2019;294(36):13248-13268. DOI: https://www.jbc.org/content/early/2019/07/26/jbc.RA119.009671.short
34. Fries, L, Cho, I, Krahling, V, Fehling, SK, Strecker, T, et al. Randomized, blinded, dose-ranging trial of an Ebola virus glycoprotein (EBOV GP) nanoparticle vaccine with Matrix-M adjuvant in healthy adults. *J Infect Dis.* 2019 Oct 11:jiz518. [Epub ahead of print]. DOI: https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiz518/5585635
35. Fukuda, H, Li, S, Sardo, L, Smith, JL, Yamashita, K, et al. Structural determinants of the APOBEC3G N-terminal domain for HIV-1 RNA association. *Front Cell Infect Microbiol.* 2019;9:129. DOI: https://www.frontiersin.org/articles/10.3389/fcimb.2019.00129/full

36. Garrison, AR, Smith, DR, Golden, JW. Animal models for Crimean-Congo hemorrhagic fever human disease. *Viruses.* 2019;11(7):E590. DOI: https://www.mdpi.com/1999-4915/11/7/590
37. Gilkes, AP, Albin, TJ, Manna, S, Supnet, M, Ruiz, S, et al. Tuning subunit vaccines with novel TLR triagonist adjuvants to generate protective immune responses against Coxiella burnetii. *J Immunol.* 2019 Dec 23:ji1900991. [Epub ahead of print]. DOI: https://www.jimmunol.org/content/early/2019/12/21/jimmunol.1900991
38. Golden, JW, Shoemaker, CJ, Lindquist, ME, Zeng, XK, Daye, SP, et al. GP38-targeting monoclonal antibodies protect adult mice against lethal Crimean-Congo hemorrhagic fever virus infection. *Sci Adv.* 2019;5(7):eaaw9535. DOI: https://advances.sciencemag.org/content/5/7/eaaw9535
39. Gomes-Solecki, M, Arnaboldi, PM, Backenson, PB, Benach, JL, Cooper, CL, et al. Protective immunity and new vaccines for Lyme disease. *Clin Infect Dis.* 2019 Oct 17:ciz872. [Epub ahead of print]. DOI: https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciz872/5588349
40. Grubaugh, ND, Saraf, S, Gangavarapu, K, Watts, A, Tan, AL, et al. Travel surveillance and genomics uncover a hidden Zika outbreak during the waning epidemic. *Cell.* 2019;178(5):1057-1071.e1011. DOI: https://www.sciencedirect.com/science/article/pii/S0092867419307834?via%3Dihub
41. Happi, AN, Happi, CT, Schoepp, RJ. Lassa fever diagnostics: past, present, and future. *Curr Opin Virol.* 2019;37:132-138. DOI: https://www.sciencedirect.com/science/article/abs/pii/S1879625719300677
42. Haston, JC, Rostad, CA, Jerris, RC, Milla, SS, McCracken, C, et al. Prospective cohort study of next-generation sequencing as a diagnostic modality for unexplained encephalitis in children. *J Pediatric Infect Dis Soc.* 2019 May 20:piz032. [Epub ahead of print]. DOI: https://academic.oup.com/jpids/advance-article-abstract/doi/10.1093/jpids/piz032/5492331?redirectedFrom=fulltext
43. Hogan, M, Bahta, M, Tsuji, K, Nguyen, TX, Cherry, S, et al. Targeting protein-protein interactions of tyrosine phosphatases with microarrayed fragment libraries displayed on phosphopeptide substrate scaffolds. *ACS Comb Sci.* 2019;21(3):158-170. DOI: https://pubs.acs.org/doi/10.1021/acscombsci.8b00122
44. Hulseberg, CE, Fénéant, L, Szymanska-De Wijs, KM, Kessler, NP, Nelson, EA, et al. Arbidol and other low-molecular-weight drugs that inhibit Lassa and Ebola viruses. *J Virol.* 2019;93(8):e02185-18. DOI: https://jvi.asm.org/content/93/8/e02185-18
45. Jasenosky, LD, Cadena, C, Mire, CE, Borisevich, V, Haridas, V, et al. The FDA-approved oral drug nitazoxanide amplifies host antiviral responses and inhibits Ebola virus. *iScience.* 2019;19:1279-1290. DOI: https://www.sciencedirect.com/science/article/pii/S2589004219302287
46. Jiang, JJ, Banglore, P, Cashman, KA, Schmaljohn, CS, Schultheis, K, et al. Immunogenicity of a protective intradermal DNA vaccine against Lassa virus in cynomolgus macaques. *Hum Vaccin Immunother.* 2019:15(9):2066-2074. DOI: https://www.tandfonline.com/doi/full/10.1080/21645515.2019.1616499
47. Katawera, V, Kohar, H, Mahmoud, N, Raftery, P, Wasunna, C, et al. Enhancing laboratory capacity during Ebola virus disease (EVD) heightened surveillance in Liberia: lessons learned and recommendations. *Pan Afr Med J.* 2019;33(suppl 2):8. DOI: https://www.panafrican-med-journal.com/content/series/33/2/8/full/
48. Kayiwa, JT, Nankya, AM, Ataliba, I, Nassuna, CA, Omara, IE, et al. Dengue fever and chikungunya virus infections: identification in travelers in Uganda - 2017. *Trop Dis Travel Med Vaccines.* 2019;5:21. DOI: https://tdtmvjournal.biomedcentral.com/articles/10.1186/s40794-019-0099-3
49. Keasey, SL, Suh, MJ, Das, S, Blancett, CD, Zeng, X, et al. Decreased antibiotic susceptibility driven by global remodeling of the klebsiella pneumoniae proteome. *Mol Cell Proteomics.* 2019;18(4):657-668. DOI: https://www.mcponline.org/content/18/4/657
50. Kende, M, Paragas, J, Salazar, AM. The efficacy of poly-ICLC against Ebola-Zaire virus (EBOV) infection in mice and cynomolgus monkeys. *Antiviral Res.* 2019;163:179-184. DOI: https://www.sciencedirect.com/science/article/abs/pii/S0166354218304078?via%3Dihub

51. Khan, M, Miller, C, Hale, M. Development of an assay for antimicrobial susceptibility testing of Coxiella burnetii. *Abstr Pap Am Chem Soc.* 2019;257. DOI: https://mdsoar.org/bitstream/handle/11603/13530/Draft%2012%20Departmental%20Honors%20Paper%20Khan%20%281%29.pdf?sequence=1&isAllowed=y
52. Kijek, TM, Mou, S, Bachert, BA, Kuehl, KA, Williams, JA, et al. The D-alanyl-D-alanine carboxypeptidase enzyme is essential for virulence in the Schu S4 strain of Francisella tularensis and a dacD mutant is able to provide protection against a pneumonic challenge. *Microb Pathog.* 2019;137: 103742. DOI: https://www.sciencedirect.com/science/article/abs/pii/S0882401019310587?via%3Dihub
53. Kim, WK, No, JS, Lee, D, Jung, J, Park, H, et al. Active targeted surveillance to identify sites of emergence of Hantavirus. *Clin Infect Dis.* 2019 Mar 20:ciz234. [Epub ahead of print]. DOI: https://www.ncbi.nlm.nih.gov/pubmed/30891596
54. Kinoshita, Y, Cloutier, AK, Rozak, DA, Khan, MSR, Niwa, H, et al. A novel selective medium for the isolation of Burkholderia mallei from equine specimens. *BMC Vet Res.* 2019;15(1):133. DOI: https://bmcvetres.biomedcentral.com/articles/10.1186/s12917-019-1874-055. Ko, SY, Akahata, W, Yang, ES, Kong, WP, Burke, CW, et al. A virus-like particle vaccine prevents equine encephalitis virus infection in nonhuman primates. *Sci Transl Med.* 2019;11(492):eaav3113. DOI: https://stm.sciencemag.org/content/11/492/eaav3113.short
56. Krakauer, T. Staphylococcal superantigens: Pyrogenic toxins induce toxic shock. *Toxins.* 2019:11(3). DOI: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6468478/
57. Krishnamurthy, M, Lemmon, MM, Falcinelli, EM, Sandy, RA, Dootz, JN, et al. Enhancing the antibacterial activity of polymyxins using a nonantibiotic drug. *Infect Drug Resist.* 2019;12:1393-1405. DOI: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6555264/
58. Kuhn, JH, Amarasinghe, GK, Basler, CF, Bavari, S, Bukreyev, A, et al. ICTV virus taxonomy profile: Filoviridae. *J Gen Virol.* 2019;100(6):911-912. DOI: https://www.ncbi.nlm.nih.gov/pubmed/31021739
59. Larson, GP, Tran, V, Yu, SQ, Cai, YY, Higgins, CA, et al. EPS8 facilitates uncoating of influenza A virus. *Cell Reports.* 2019;29(8):2175-2183.e4. DOI: https://www.sciencedirect.com/science/article/pii/S2211124719313786?via%3Dihub
60. Lindquist, ME, Hicar, MD. B Cells and antibodies in Kawasaki disease. *Int J Mol Sci.* 2019;20(8):E1834. DOI: https://www.mdpi.com/1422-0067/20/8/1834/htm
61. Lin, J, Coffin, KM, Johnston, SC, Babka, AM, Bell, TM, et al. Nipah virus persists in the brains of nonhuman primate survivors. *JCI Insight.* 2019:4(14):129629. DOI: https://insight.jci.org/articles/view/129629
62. Maes, P, Adkins, S, Alkhovsky, SV, Avšič-Županc, T, Ballinger, MJ, et al. Taxonomy of the order Bunyavirales: second update 2018. *Arch Virol.* 2019;164(3):927-941. DOI: https://link.springer.com/article/10.1007%2Fs00705-018-04127-3
63. Maes, P, Amarasinghe, GK, Ayllón, MA, Basler, CF, Bavari, S, et al. Taxonomy of the order Mononegavirales: second update 2018. *Arch Virol.* 2019;164(4):1233-1244. DOI: https://link.springer.com/article/10.1007%2Fs00705-018-04126-4
64. Martins, KA, Gregory, MK, Valdez, SM, Sprague, TR, Encinales, L, et al. Neutralizing antibodies from convalescent chikungunya virus patients can cross-neutralize Mayaro and Una Viruses. *Am J Trop Med Hyg.* 2019;100(6):1541-1544. DOI: https://www.ncbi.nlm.nih.gov/pubmed/31017081
65. Mbala-Kingebeni, P, Aziza, A, Di Paola, N, Wiley, MR, Makiala-Mandanda, S, et al. Medical countermeasures during the 2018 Ebola virus disease outbreak in the North Kivu and Ituri Provinces of the Democratic Republic of the Congo: a rapid genomic assessment. *Lancet Infect Dis.* 2019;19(6):648-657. DOI: https://www.sciencedirect.com/science/article/pii/S1473309919301185?via%3Dihub
66. Mbala-Kingebeni, P, Pratt, CB, Wiley, MR, Diagne, MM, Makiala-Mandanda, S, et al. 2018 Ebola virus disease outbreak in Équateur Province, Democratic Republic of the Congo: a retrospective

genomic characterisation. *Lancet Infect Dis.* 2019;19(6):641-647. DOI: https://www.thelancet.com/pdfs/journals/laninf/PIIS1473-3099(19)30124-0.pdf
67. McCall, S, Kreindl, G, Kastinger, T, Muller, E, Zahrer, W, et al. Rudolf Hess - the Doppelganger conspiracy theory disproved. *Forensic Sci Int Genet.* 2019;40:18-22. DOI: https://www.sciencedirect.com/science/article/abs/pii/S1872497317302971?via%3Dihub
68. Miller, CN, Khan, M, Ahmed, SA, Kota, K, Panchal, RG, Hale, ML. Development of a Coxiella burnetii culture method for high-throughput assay to identify host-directed therapeutics. *J Microbiol Methods.* 2019 Dec 17;169:105813-105813. [Epub ahead of print]. DOI: https://www.sciencedirect.com/science/article/pii/S0167701219310085?via%3Dihub
69. Mohamed, YF, Scott, NE, Molinaro, A, Creuzenet, C, Ortega, X, et al. A general protein O-glycosylation machinery conserved in Burkholderia species improves bacterial fitness and elicits glycan immunogenicity in humans. *J Biol Chem.* 2019;294(36):13248-13268. DOI: https://www.jbc.org/content/early/2019/07/26/jbc.RA119.009671.abstract
70. Morazzani, EM, Compton, JR, Leary, DH, Berry, AV, Hu, X, et al. Proteolytic cleavage of host proteins by the Group IV viral proteases of Venezuelan equine encephalitis virus and Zika virus. *Antiviral Res.* 2019;164:106-122. DOI: https://www.sciencedirect.com/science/article/pii/S016635421830603X?via%3Dihub
71. Morwitzer, MJ, Tritsch, SR, Cazares, LH, Ward, MD, Nuss, JE, Bavari, S, Reid, SP. Identification of RUVBL1 and RUVBL2 as novel cellular interactors of the Ebola virus nucleoprotein. *Viruses.* 2019;11(4):E372. DOI: https://www.mdpi.com/1999-4915/11/4/372/htm
72. Nalca, A, Totura, A, Livingston, V, Frick, O, Dyer, D. African green monkey model of Middle East respiratory syndrome coronavirus (MERS-CoV) infection. *Int J Infect Dis.* 2019;79(suppl 1):99-100. DOI: https://www.ijidonline.com/article/S1201-9712(18)34828-8/fulltext
73. Naluyima, P, Kayondo, W, Ritchie, C, Wandege, J, Kagabane, S, et al. The Joint Mobile Emerging Disease Clinical Capability (JMEDICC) laboratory approach: capabilities for high-consequence pathogen clinical research. *PLoS Negl Trop Dis.* 2019;13(12):e0007787. DOI: https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0007787
74. Natesan, M, Wu, SW, Chen, CI, Jensen, SMR, Karlovac, N, et al. A Smartphone-Based Rapid Telemonitoring System for Ebola and Marburg Disease Surveillance. *Acs Sens.* 2019;4(1):61-68. DOI: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6350200/
75. Nimo-Paintsil, SC, Fichet-Calvet, E, Borremans, B, Letizia, AG, Mohareb, E, et al. Rodent-borne infections in rural Ghanaian farming communities [published correction appears in *PLoS One.* 2019;14(6):e0218271]. *PLoS ONE.* 2019;14(4):e0215224. DOI: https://www.ncbi.nlm.nih.gov/pubmed/31017931
76. No, JS, Kim, WK, Cho, S, Lee, SH, Kim, JA, et al. Comparison of targeted next-generation sequencing for whole-genome sequencing of Hantaan orthohantavirus in Apodemus agrarius lung tissues. *Sci Rep.* 2019;9(1):16631. DOI: https://www.nature.com/articles/s41598-019-53043-2
77. Olson, MA, Legler, PM, Zabetakis, D, Turner, KB, Anderson, GP, Goldman, ER. Sequence tolerance of a single-domain antibody with a high thermal stability: comparison of computational and experimental fitness profiles. *ACS Omega.* 2019;4(6):10444-10454. DOI: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6648363/
78. Pandya, UM, Egbuta, C, Norman, TMA, Chiang, CY, Wiersma, VR, et al. The biophysical interaction of the danger-associated molecular pattern (DAMP) calreticulin with the pattern-associated molecular pattern (PAMP) lipopolysaccharide. *Int J Mol Sci.* 2019;20(2):E408. DOI: https://www.mdpi.com/1422-0067/20/2/408/htm
79. Perez-Sautu, U, Wiley, MR, Iglesias-Caballero, M, Pozo, F, Prieto, K, et al. Target-independent high-throughput sequencing methods provide evidence that already known human viral pathogens play a main role in respiratory infections with unexplained etiology. *Emerg Microbes Infect.* 2019;8(1):1054-1065. DOI: https://www.tandfonline.com/doi/full/10.1080/22221751.2019.1640587

80. Perley, CC, Brocato, RL, Kwilas, SA, Daye, S, Moreau, A, et al. Three asymptomatic animal infection models of hemorrhagic fever with renal syndrome caused by hantaviruses. *PLoS One.* 2019;14(5):e0216700. DOI: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0216700
81. Pessi, A, Bixler, SL, Soloveva, V, Radoshitzky, S, Retterer, C, et al. Cholesterol-conjugated stapled peptides inhibit Ebola and Marburg viruses in vitro and in vivo. *Antiviral Res.* 2019;171:104592. DOI: https://www.sciencedirect.com/science/article/pii/S0166354219302992?via%3Dihub
82. Philip, CO, Koech, M, Kipkemoi, N, Kirera, R, Ndonye, J, et al. Evaluation of the performance of a multiplex reverse transcription polymerase chain reaction kit as a potential diagnostic and surveillance kit for rotavirus in Kenya. *Trop Dis Travel Med Vaccines.* 2019;5:12. DOI: https://tdtmvjournal.biomedcentral.com/articles/10.1186/s40794-019-0087-7
83. Pittman, PR, Hahn, M, Lee, HS, Koca, C, Samy, N, et al. Phase 3 efficacy trial of modified vaccinia Ankara as a vaccine against smallpox. *N Engl J Med.* 2019;381(20):1897-1908. DOI: https://www.nejm.org/doi/full/10.1056/NEJMoa1817307
84. Pleet, ML, DeMarino, C, Stonier, SW, Dye, JM, Jacobson, S, Aman, MJ, Kashanchi, F. Extracellular vesicles and ebola virus: A new mechanism of immune evasion. *Viruses.* 2019;11(5):E410. DOI: https://www.mdpi.com/1999-4915/11/5/410/htm
85. Prescott, J, Guito, JC, Spengler, JR, Arnold, CE, Schuh, AJ, et al. Rousette bat dendritic cells overcome Marburg virus-mediated antiviral responses by upregulation of interferon-related genes while downregulating proinflammatory disease mediators. *mSphere.* 2019;4(6):e00728-19. DOI: https://msphere.asm.org/content/4/6/e00728-19
86. Quiroz, JA, Malonis, RJ, Thackray, LB, Cohen, CA, Pallesen, J, et al. Human monoclonal antibodies against chikungunya virus target multiple distinct epitopes in the E1 and E2 glycoproteins. *PLoS Pathog.* 2019;15(11):e1008061. DOI: https://journals.plos.org/plospathogens/article?id=10.1371%2Fjournal.ppat.1008061
87. Rajamani, S, Sandy, R, Kota, K, Lundh, L, Gomba, G, et al. Robust biofilm assay for quantification and high throughput screening applications. *J Microbiol Methods.* 2019;159:179-185. DOI: https://www.sciencedirect.com/science/article/pii/S0167701219300296
88. Ramirez de Arellano, E, Sanchez-Lockhart, M, Perteguer, MJ, Bartlett, M, Ortiz, M, et al. First evidence of antibodies against Lloviu virus in Schreiber's bent-winged insectivorous bats demonstrate a wide circulation of the virus in Spain. *Viruses.* 2019;11(4):E360. DOI: https://www.mdpi.com/1999-4915/11/4/360/htm
89. Ricks, KM, Koehler, J, Shoemaker, C, Voorhees, M, Schoepp, R. Development of a sustainable diagnostic toolbox for serosurveillance of West African infectious diseases. *Int J Infect Dis.* 2019;79(suppl 1):24-25. DOI: https://www.sciencedirect.com/science/article/pii/S1201971218346538
90. Ricks, KM, Shoemaker, CJ, Dupuy, LC, Flusin, O, Voorhees, MA, et al. Development of a bead-based immunoassay using virus-like particles for detection of alphaviral humoral response. *J Virol Methods.* 2019;270:12-17. DOI: https://www.sciencedirect.com/science/article/abs/pii/S0166093418305421
91. Rusnak, JM, Glass, PJ, Weaver, SC, Sabourin, CL, Glenn, AM, et al. Approach to strain selection and the propagation of viral stocks for Venezuelan equine encephalitis virus vaccine efficacy testing under the Animal Rule. *Viruses.* 2019;11(9):E807. DOI: https://www.mdpi.com/1999-4915/11/9/807/htm
92. Salami, K, Gouglas, D, Schmaljohn, C, Saville, M, Tornieporth, N. A review of Lassa fever vaccine candidates. *Curr Opin Virol.* 2019;37:105-111. DOI: https://www.sciencedirect.com/science/article/abs/pii/S1879625719300501?via%3Dihub
93. Samsa, MM, Dupuy, LC, Beard, CW, Six, CM, Schmaljohn, CS, et al. Self-amplifying RNA vaccines for Venezuelan equine encephalitis virus induce robust protective Immunogenicity in mice. *Mol Ther.* 2019;27(4):850-865. DOI: https://www.sciencedirect.com/science/article/abs/pii/S1525001619300012?via%3Dihub

94. Scarff, JM, Waidyarachchi, SL, Meyer, CJ, Lane, DJ, Chai, W, et al. Aminomethyl spectinomycins: a novel antibacterial chemotype for biothreat pathogens. *J Antibiot (Tokyo).* 2019;72(9):693-701. DOI: https://www.nature.com/articles/s41429-019-0194-8
95. Schmaljohn, C, Safronetz, D. Editorial overview: Lassa virus. *Curr Opin Virol.* 2019;37:vii-ix. DOI: https://www.sciencedirect.com/science/article/abs/pii/S1879625719300835?via%3Dihub
96. Schubert, SL, Melanson, VR. Prevalence of Lyme disease attributable to military service at the USMA, West Point NY: FY2016-2018. *Mil Med.* 2019 Jun 27:usz156. [Epub ahead of print]. DOI: https://academic.oup.com/milmed/article/185/1-2/e28/5524322
97. Shearer, JD, Saylor, ML, Butler, CM, Treston, AM, Heine, HS, et al. GC-072: A novel therapeutic candidate for oral treatment of melioidosis and infections caused by select biothreat pathogens. *Antimicrob Agents Chemother.* 2019;63(12):e00834-19. DOI: https://aac.asm.org/content/63/12/e00834-19.long
98. Sichtig, H, Minogue, T, Yan, Y, Stefan, C, Hall, A, et al. FDA-ARGOS is a database with public quality-controlled reference genomes for diagnostic use and regulatory science. *Nat Commun.* 2019;10(1):3313. DOI: https://www.nature.com/articles/s41467-019-11306-6
99. Siddharthan, V, Miao, J, Van Wettere, AJ, Li, R, Wu, H, et al. Human polyclonal antibodies produced from transchromosomal bovine provides prophylactic and therapeutic protections against zika virus infection in STAT2 KO Syrian hamsters. *Viruses.* 2019;11(2):E92. DOI: https://www.mdpi.com/1999-4915/11/2/92/htm
100. Smith, D, Shoemaker, CJ, Garrison, A, Ricks, KM, Flusin, O, et al. The pathogenesis of genetically diverse strains of Crimean-Congo hemorrhagic fever virus in the cynomolgus macaque model. *Int J Infect Dis.* 2019;79(suppl 1):16-16. DOI: https://www.sciencedirect.com/science/article/pii/S1201971218346356
101. Smith, DR, Shoemaker, CJ, Zeng, X, Garrison, AR, Golden, JW, et al. Persistent Crimean-Congo hemorrhagic fever virus infection in the testes and within granulomas of non-human primates with latent tuberculosis. *PLoS Pathog.* 2019;15(9):e1008050. DOI: https://journals.plos.org/plospathogens/article?id=10.1371%2Fjournal.ppat.1008050
102. Sobarzo, A, Stonier, SW, Radinsky, O, Gelkop, S, Kuehne, AI, et al. Multiple viral proteins and immune response pathways act to generate robust long-term immunity in Sudan virus survivors. *EBioMedicine.* 2019;46:215-226. DOI: https://www.sciencedirect.com/science/article/pii/S2352396419304578?via%3Dihub
103. Steger, CL, Brown, ML, Sullivan, OM, Boudreaux, CE, Cohen, CA, LaConte, LEW, McDonald, SM. In vitro double-stranded RNA synthesis by rotavirus polymerase mutants with lesions at core shell contact sites. *J Virol.* 2019;93(20):e01049-19. DOI: https://jvi.asm.org/content/93/20/e01049-19.long
104. Suschak, JJ, Schmaljohn, CS. Vaccines against Ebola virus and Marburg virus: recent advances and promising candidates. *Hum Vaccin Immunother.* 2019;15(10):2359-2377. DOI: https://www.tandfonline.com/doi/full/10.1080/21645515.2019.1651140
105. Totura, AL, Bavari, S. Broad-spectrum coronavirus antiviral drug discovery. *Expert Opin Drug Discov.* 2019;14(4):397-412. DOI: https://www.tandfonline.com/doi/full/10.1080/17460441.2019.1581171
106. Trobaugh, DW, Sun, CQ, Bhalla, N, Gardner, CL, Dunn, M, Klimstra, WB. Cooperativity between the 3' untranslated region microRNA binding sites is critical for the virulence of eastern equine encephalitis virus. *PLoS Pathog.* 2019;15(10):e1007867. DOI: https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1007867
107. Turell, MJ, Gozalo, AS, Guevara, C, Schoeler, GB, Carbajal, F, Lopez-Sifuentes, VM, Watts, DM. Lack of evidence of sylvatic transmission of dengue viruses in the Amazon Rainforest near Iquitos, Peru. *Vector Borne Zoonotic Dis.* 2019;19(9):685-689. DOI: https://www.liebertpub.com/doi/full/10.1089/vbz.2018.2408?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub%3Dpubmed&

108. Turner, MJ, Cox, JK, Spellman, AC, Stahl, C, Bavari, S. Avoidance behavior independent of innate-immune signaling seen in Caenorhabditis elegans challenged with Bacillus anthracis. *Dev Comp Immunol.* 2020;102:103453. DOI: https://www.sciencedirect.com/science/article/pii/S0145305X18304579?via%3Dihub
109. Verdonck, S, Pu, SY, Sorrell, FJ, Elkins, JM, Froeyen, M, et al. Synthesis and structure-activity relationships of 3,5-disubstituted-pyrrolo 2,3-b pyridines as inhibitors of adaptor-associated kinase 1 with antiviral activity. *J Med Chem.* 2019;62(12):5810-5831. DOI: https://pubs.acs.org/doi/10.1021/acs.jmedchem.9b00136
110. von Fricken, ME, Qurollo, BA, Boldbaatar, B, Wang, YW, Jiang, RR, et al. Genetic diversity of Anaplasma and Ehrlichia bacteria found in Dermacentor and Ixodes ticks in Mongolia. *Ticks Tick Borne Dis.* 2020;11(1):101316. DOI: https://www.sciencedirect.com/science/article/pii/S1877959X18304771?via%3Dihub
111. Voorhees, M, Ricks, KM, Fulmer, A, Quesinberry, J, Poli, M, Schoepp, R. Development of a multiplexed antigen detection immunoassay for detection of viral agents. *Int J Infect Dis.* 2019;79(suppl 1):106-106. DOI: https://www.ijidonline.com/article/S1201-9712(18)34843-4/fulltext
112. Ward, MD, Brueggemann, EE, Kenny, T, Reitstetter, RE, Mahone, CR, et al. Characterization of the plasma proteome of nonhuman primates during Ebola virus disease or melioidosis: a host response comparison. *Clin Proteomics.* 2019;16:7. DOI: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6366079/
113. Wec, AZ, Bornholdt, ZA, He, S, Herbert, AS, Goodwin, E, et al. Development of a human antibody cocktail that deploys multiple functions to confer pan-Ebolavirus protection. *Cell Host Microbe.* 2019;25(1):39-48.e35. DOI: https://www.sciencedirect.com/science/article/pii/S1931312818306310?via%3Dihub
114. West, BR, Wec, AZ, Moyer, CL, Fusco, ML, Ilinykh, PA, et al. Structural basis of broad ebolavirus neutralization by a human survivor antibody. *Nat Struct Mol Biol.* 2019;26(3):204-212. DOI: https://www.nature.com/articles/s41594-019-0191-4
115. Wiley, MR, Fakoli, L, Letizia, AG, Welch, SR, Ladner, JT, et al. Lassa virus circulating in Liberia: a retrospective genomic characterisation. *Lancet Infect Dis.* 2019;19(12):1371-1378. DOI: https://www.sciencedirect.com/science/article/abs/pii/S1473309919304864?via%3Dihub
116. Williams, MS, Baker, MR, Guina, T, Hewitt, JA, Lanning, L, et al. Retrospective analysis of pneumonic tularemia in Operation Whitecoat human subjects: disease progression and tetracycline efficacy. *Front Med (Lausanne).* 2019;6:229. DOI: https://www.frontiersin.org/articles/10.3389/fmed.2019.00229/full
117. Williamson, LE, Flyak, AI, Kose, N, Bombardi, R, Branchizio, A, et al. Early human B cell response to Ebola virus in four U.S. survivors of infection. *J Virol.* 2019;93(8):e01439-18. DOI: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6450119/
118. Yeh, KB, Wood, H, Scullion, M, Russell, JA, Parker, K, Gnade, BT, Jones, AR, Whittier, C, Mereish, K. Molecular detection of biological agents in the field: then and now. *mSphere.* 2019;4(6):e00695-19. DOI: https://msphere.asm.org/content/4/6/e00695-19
119. Zabetakis, D, Shriver-Lake, LC, Olson, MA, Goldman, ER, Anderson, GP. Experimental evaluation of single-domain antibodies predicted by molecular dynamics simulations to have elevated thermal stability. *Protein Sci.* 2019;28(10):1909-1912. DOI: https://onlinelibrary.wiley.com/doi/full/10.1002/pro.3692

5. Briefly describe the biological defence work carried out at the facility, including type(s) of microorganisms* and/or toxins studied, as well as outdoor studies of biological aerosols:

* Including viruses and prions.

Objectives: USAMRIID develops medical countermeasures, including candidate vaccines, diagnostic tests and drug or immunological therapies for biological agents, and performs exploratory studies and advanced development of protective and therapeutic countermeasures and agent identification technologies. Additional information is available at http://www.usamriid.army.mil/.

Agents Microorganisms and/or Toxins: Select Agents and Toxins (HHS and Overlap Select Agents, NIAID Category A pathogens, and HHS Select Toxins)

Outdoor Studies: None

Form A, Part 2 (iii)

National biological defence research and development programmes: Facilities

1. What is the name of the facility?
Argonne National Laboratory (ANL). Argonne National Laboratory initiated a biological defense research and development project in 2019 that requires a BSL-2 laboratory.

(b)(5)

2. Where is it located (provide both address and geographical location)?
9700 South Cass Ave., Lemont, IL 60439
(Located 41 km southwest of Chicago, Illinois)

3. Floor area of laboratory areas by containment level (m^2):

BSL-2:	28 m^2
BSL-3:	0 m^2
BSL-4:	0 m^2
Total laboratory floor area:	28 m^2

4. The organizational structure of each facility:

(i) Total number of personnel: 5

(ii) Division of personnel:

Military	0
Civilian	5

(iii) Division of personnel by category:

Scientists	5
Engineers	0
Technicians	0
Administrative and support staff	0

(iv) List the scientific disciplines represented in the scientific/engineering staff:
Biotechnology

(v) Are contractor staff working in the facility? If so, provide an approximate number:
No

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
Internal: Laboratory Directed Research and Development (LDRD)

(vii) What are the funding levels for the following program areas:

Research	$97,000
Development	$0
Test and evaluation	$0
Total	$97,000

(viii) Briefly describe the publication policy of the facility:
As a Department of Energy facility, ANL is required to make scientific and technical information broadly available, within applicable laws and Departmental requirements, to accomplish mission objectives and

strategic goals, promote scientific advancement, satisfy statutory dissemination requirements, and ensure a fair return on Departmental and taxpayer investment. ANL has a mandate to ensure that scientific and technical information is identified, processed, disseminated, and preserved to enable the scientific community and the public to locate and use the unclassified and unlimited-distribution information resulting from DOE research and related endeavors. ANL also has procedures in place to manage and protect classified, sensitive controlled unclassified, and export-controlled scientific and technical information, yet make it accessible for appropriate access by the Department, its contractors, and others. Reviews are conducted prior to publication to determine availability of information, or restrictions thereto. These reviews include, but are not limited to, the following: 1) classification/declassification, 2) copyrighted materials or other intellectual property, 3) export controls or distribution restrictions, and 4) sensitive content that limits access. [US Department of Energy, Scientific and Technical Information Management: https://www.directives.doe.gov/directives/0241.1-BOrder-b/view]

(ix) Provide a list of publicly-available papers and reports resulting from the work during the previous 12 months. (To include authors, titles, and full references.):

None

5. Briefly describe the biological defence work carried out at the facility, including type(s) of microorganisms* and/or toxins studied, as well as outdoor studies of biological aerosols:

Objectives: The biological defense research conducted at Argonne National Laboratory includes research on printed biosensors aims to rapid prototype highly sensitive, multiplexed, label-free biosensors that can effectively detect and persistently monitor toxic and biological weapon (CBW) agents.

Microorganisms and/or toxins studied: None

Outdoor Studies: None

* Including viruses and prions.

Form A, Part 2 (iii)

National biological defence research and development programmes: Facilities

1. What is the name of the facility?
Lawrence Livermore National Laboratory (LLNL)

2. Where is it located (provide both address and geographical location)?
7000 East Avenue, Livermore, California 94550
(Located 62 km east-southeast of San Francisco, California)

3. Floor area of laboratory areas by containment level (m^2):

BSL-2:	1,822.5 m^2
BSL-3:	59.5 m^2
BSL-4:	0 m^2
Total laboratory floor area:	1,882 m^2

Note: The decrease in BSL-2 lab space of approximately 10 m^2 from 2018 to 2019 is due to a BSL-2 laboratory undergoing renovation during 2019 and not being used for research as well as refinements made in how space is counted.

4. The organizational structure of each facility:

(i) Total number of personnel: 57

(ii) Division of personnel:

Military:	0
Civilian:	57

(iii) Division of personnel by category:

Scientists	19
Engineers	14
Technicians	11
Administrative and support staff	13

(iv) List the scientific disciplines represented in the scientific/engineering staff:
Aerosol Science, Analytical Biochemistry, Analytical Mass Spectrometry, Bacteriology, Biochemistry, Bioinformatics, Biomedical Engineering, Biomedical Science, Biotechnology, Computational Biology, Computer Science, Environmental Science, Epidemiology, Genomics, Immunology, Mass Spectrometry, Microbial Forensics, Microbiology, Molecular Biology, Molecular Diagnostics, Nanotechnology, Proteomics, Toxinology, Virology.

(v) Are contractor staff working in the facility? If so, provide an approximate number:
No

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
U.S. Department of Defense (DoD) – partially
U.S. Department of Energy (DOE)
U.S. Department of Homeland Security (DHS)
Internal (Laboratory Directed Research and Development)

(vii) What are the funding levels for the following program areas:

Research	$ 1,966,000
Development	$ 1,640,000
Test and evaluation	$ 483,000
Total	$ 4,089,000

(viii) Briefly describe the publication policy of the facility:

As a Department of Energy facility, LLNL is required to make scientific and technical information broadly available, within applicable laws and Departmental requirements, to accomplish mission objectives and strategic goals, promote scientific advancement, satisfy statutory dissemination requirements, and ensure a fair return on Departmental and taxpayer investment. LLNL has a mandate to ensure that scientific and technical information is identified, processed, disseminated, and preserved to enable the scientific community and the public to locate and use the unclassified and unlimited-distribution information resulting from DOE research and related endeavors. LLNL also has procedures in place to manage and protect classified, sensitive controlled unclassified, and export-controlled scientific and technical information, yet make it accessible for appropriate access by the Department, its contractors, and others. Reviews are conducted prior to publication to determine availability of information, or restrictions thereto. These reviews include, but are not limited to, the following: 1) classification/declassification, 2) copyrighted materials or other intellectual property, 3) export controls or distribution restrictions, and 4) sensitive content that limits access. [US Department of Energy, Scientific and Technical Information Management: https://www.directives.doe.gov/directives/0241.1-BOrder-b/view]

(ix) Provide a list of publicly-available papers and reports resulting from the work during the previous 12 months. (To include authors, titles, and full references.):

1. Kane SR, Shah SR, Alfaro TM. Development of a rapid viability polymerase chain reaction method for detection of *Yersinia pestis*. Journal of Microbiological Methods. 2019. 162, July 2019, Pages 21-27. https://doi.org/10.1016/j.mimet.2019.05.005
2. Kane SR, Sanjiv R. Shah SR, Alfaro TM. Rapid viability polymerase chain reaction method for detection of *Francisella tularensis*. Journal of Microbiological Methods. 2019. https://doi.org/10.1016/j.mimet.2019.105738
3. Martin E, Borucki M, Thissen J, et al. Mosquito-Borne Viruses and Insect-Specific Viruses Revealed in Field-Collected Mosquitoes by a Monitoring Tool Adapted from a Microbial Detection Array. Appl Environ Microbiol 2019 Oct 17;85(19). Epub 2019 Sep 17. doi: 10.1128/AEM.0120219. https://www.ncbi.nlm.nih.gov/pubmed/31350319
4. Prentice KW, DePalma L, Ramage JG, et al. Comprehensive Laboratory Evaluation of a Lateral Flow Assay for the Detection of *Yersinia pestis*. Health Security. 2019; 17(6)439-453. DOI: 10.1089/hs.2019.0094l. https://www.liebertpub.com/doi/pdf/10.1089/hs.2019.0094

5. Briefly describe the biological defence work carried out at the facility, including type(s) of microorganisms[*] and/or toxins studied, as well as outdoor studies of biological aerosols:

Objectives: The biological defense research conducted at Lawrence Livermore National Laboratory includes biological agent detection, therapeutics and prophylactics development, bioinformatics, virulence mechanism elucidation, structural characterization, agent viability testing, response planning, assay development for monitoring for biological decontamination/response as well as microbial forensic

[*] Including viruses and prions.

assay development to help determine geographic origin and attribution. LLNL also works to develop diagnostic platforms that use a variety of techniques, such as polymerase chain reaction (PCR), immunoassay, microarray, mass spectrometry and genomic sequencing used to gather useful information about the species present in the sampling environment. Beyond detection, response, recovery, and attribution, LLNL also has ongoing research projects to elucidate mechanisms of host-pathogen interactions. Additional information is available at https://st.llnl.gov/.

Microorganisms and/or Toxins Studied: Select Agents (HHS, Overlap), NIAID Category A pathogens, HHS Select Toxins.

Outdoor Studies: None.

Form A, Part 2 (iii)

National biological defence research and development programmes: Facilities

1. What is the name of the facility?
Los Alamos National Laboratory (LANL)

2. Where is it located (provide both address and geographical location)?
Bikini Atoll Road, SM-30, Los Alamos, NM 87545
(Located approximately 72 km west of Santa Fe, New Mexico)

3. Floor area of laboratory areas by containment level (m^2):

BSL-2:	533.2 m^2
BSL-3:	0 m^2
BSL-4:	0 m^2
Total laboratory floor area:	533.2 m^2

Note: Due to increased project scope in 2019, the BSL-2 laboratory space used for biodefense research and development was expanded from 354 to 533.2 m^2. A portion of the reported BSL-2 laboratory space (37.2 m^2) was physically remodeled.

(b)(5)

4. The organizational structure of each facility:

(i) Total number of personnel: 23

(ii) Division of personnel:

Military	0
Civilian	23

(iii) Division of personnel by category:

Scientists	15
Engineers	0
Technicians	8
Administrative and support staff	0

(iv) List the scientific disciplines represented in the scientific/engineering staff:
Analytical Biochemistry, Bacteriology, Bioinformatics, Biomedical Science, Biological Science, Biomedical Engineering, Cell Biology, Environmental Science, Genetics, Genomics, Microbiology, Microscopy, Molecular Biology, Biomedical Science, Medicine, Toxicology, Virology.

(v) Are contractor staff working in the facility? If so, provide an approximate number:
No

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
U.S. Department of Defense (DoD) – partially
U.S. Department of Energy (DOE)
U.S. Department of Health & Human Services (HHS)
Internal (Laboratory Directed Research and Development)
Other Government Agencies

(vii) What are the funding levels for the following program areas:

Research	$2,788,000
Development	$895,000
Test and evaluation	$0
Total	$3,683,000

(viii) Briefly describe the publication policy of the facility:

As a Department of Energy facility, LANL is required to make scientific and technical information broadly available, within applicable laws and Departmental requirements, to accomplish mission objectives and strategic goals, promote scientific advancement, satisfy statutory dissemination requirements, and ensure a fair return on Departmental and taxpayer investment. LANL has a mandate to ensure that scientific and technical information is identified, processed, disseminated, and preserved to enable the scientific community and the public to locate and use the unclassified and unlimited-distribution information resulting from DOE research and related endeavors. LANL also has procedures in place to manage and protect classified, sensitive controlled unclassified, and export-controlled scientific and technical information, yet make it accessible for appropriate access by the Department, its contractors, and others. Reviews are conducted prior to publication to determine availability of information, or restrictions thereto. These reviews include, but are not limited to, the following: 1) classification/declassification, 2) copyrighted materials or other intellectual property, 3) export controls or distribution restrictions, and 4) sensitive content that limits access. [US Department of Energy, Scientific and Technical Information Management: https://www.directives.doe.gov/directives/0241.1-BOrder-b/view]

(ix) Provide a list of publicly-available papers and reports resulting from the work during the previous 12 months. (To include authors, titles, and full references.):

1. Ross B, Micheva-Viteva S, Hong-Geller E, and Torres A. Evaluating the role of *Burkholderia pseudomallei* K96243 toxins BPSS0390, BPSS0395, and BPSS1584 in persistent infection. Cellular Microbiology. 2019 Aug 10; https://doi.org/10.1111/cmi.13096
2. Kalb D, Adikari S, Hong-Geller E, and Werner JH. Single cell correlations of mRNA and protein content in human monocytic cell line after LPS stimulation. 2019 Apr 19; PLoS One 14(4): e0215602. https://doi.org/10.1371/journal.pone.0215602
3. Arzt J, Fish I, Pauszek SJ, et al. The evolution of a super-swarm of *Foot-and-mouth disease virus* in cattle. PLoS One. 2019 Apr 25; 14(4) e0210847. https://doi.org/10.1371/journal.pone.0210847.
4. Hovde BT, Daligault HE, Hanschen ER, et al. Detection of Abrin-Like and Prepropulchellin-Like Toxin Genes and Transcripts Using Whole Genome Sequencing and Full-Length Transcript Sequencing of *Abrus precatorius*. Toxins. 2019 Oct 10; 11(12):691. https://doi.org/10.3390/toxins11120691

5. Briefly describe the biological defence work carried out at the facility, including type(s) of microorganisms[*] and/or toxins studied, as well as outdoor studies of biological aerosols:

***Objectives*:** The biological defense research and development activities at the Los Alamos National Laboratory include pathogen characterization, host-pathogen interaction studies, pathogen detection, integrative biosurveillance and analysis technology development. The main objectives for the studies are to: understand molecular mechanisms of host-pathogen interaction; study molecular, chemical, and physical characteristics of biothreat agents, including bacteria, viruses and toxins, for detection,

[*] Including viruses and prions.

characterization, assay design and improvement; evaluate detection assay and platform performance; assess commercial techniques for pathogen detection and biosurveillance on environmental monitoring procedures; develop DNA, RNA and protein based bioforensics assays; develop next generation high throughput microbial sequencing, finishing and analysis capabilities; perform viral and bacterial pathogen sequencing for characterization, comparative genomic analysis, and metagenomic analysis; develop high throughput assays for host-pathogen protein interactions screening; develop and validate assays to improve the ability to identify and characterize bioterrorism incident; study antibiotic potentials of radioisotopes; and identify host molecular targets as potential therapeutic candidates. Additional information is available at https://www.lanl.gov/org/ddste/aldcels/bioscience/biosecurity-public-health/index.php.

***Microorganisms and/or Toxins Studied*:** Simulant, Overlap Select Agent and HHS Select Toxin.

***Outdoor Studies*:** None.

Form A, Part 2 (iii)

National biological defence research and development programmes: Facilities

1. What is the name of the facility?
Pacific Northwest National Laboratory (PNNL)

2. Where is it located (provide both address and geographical location)?
Personnel and budget were shared between two PNNL campuses in 2019:

Richland Campus: 902 Battelle Boulevard, Richland, Washington 99352.
(Located 235 km southwest from Spokane, WA and 327 km southeast from Seattle, WA.)

Sequim campus: 1529 West Sequim Bay Road, Sequim, Washington 98382.
(Located 489 km northwest from the PNNL Richland, WA campus and 106 km west from Seattle, WA.)

3. Floor area of laboratory areas by containment level (m^2):

Richland campus:	
BSL-2:	1470 m^2
BSL-3:	0 m^2
BSL-4:	0 m^2
Total laboratory floor area:	1470 m^2

Sequim campus:	
BSL-2:	109 m^2
BSL-3:	0 m^2
BSL-4:	0 m^2
Total laboratory floor area:	109 m^2

Note: Due to increased project scope in 2019, the BSL-2 laboratory space used for biodefense research and development was expanded from 1252 to 1470 m^2. The BSL-2 laboratory space was not physically remodeled.

4. The organizational structure of each facility:

(i) Total number of personnel: 127
Richland & Sequim campuses (shared personnel)

(ii) Division of personnel:

Military	0
Civilian	127

(iii) Division of personnel by category:

Scientists	110
Engineers	1
Technicians	0
Admin and Support Staff	16

(iv) List the scientific disciplines represented in the scientific/engineering staff:
Analytical Mass Spectrometry, Bacteriology, Biochemistry, Biological Science, Cell Biology, Chemistry, Computational Biology, Genetics, Genomics, Mass Spectrometry, Microbial Forensics, Microbiology,

Molecular Biology, Nanotechnology, Pathology, Proteomics, Structural Biology, Systems Biology, Virology.

(v) Are contractor staff working in the facility? If so, provide an approximate number:
Yes Number: 1

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
U.S. Department of Defense (DoD) - partially
U.S. Department of Energy (DOE)
U.S. Department of Homeland Security (DHS)
U.S. Department of State (DOS)
U.S. Department of Health and Human Services (HHS)
Internal (Laboratory Directed Research and Development)
Other Government Agencies

(vii) What are the funding levels for the following program areas:

Research	$10,802,512
Development	$659,064
Test and evaluation	$990,458
Total	$12,452,034

(viii) Briefly describe the publication policy of the facility:
As a Department of Energy facility, PNNL is required to make scientific and technical information broadly available, within applicable laws and Departmental requirements, to accomplish mission objectives and strategic goals, promote scientific advancement, satisfy statutory dissemination requirements, and ensure a fair return on Departmental and taxpayer investment. PNNL has a mandate to ensure that scientific and technical information is identified, processed, disseminated, and preserved to enable the scientific community and the public to locate and use the unclassified and unlimited-distribution information resulting from DOE research and related endeavors. PNNL also has procedures in place to manage and protect classified, controlled unclassified, and export-controlled scientific and technical information, yet make it accessible for appropriate access by the Department, its contractors, and others. Reviews are conducted prior to publication to determine availability of information, or restrictions thereto. These reviews include, but are not limited to, the following: 1) classification/declassification, 2) copyrighted materials or other intellectual property, 3) export controls or distribution restrictions, and 4) sensitive content that limits access. [US Department of Energy, Scientific and Technical Information Management: https://www.directives.doe.gov/directives/0241.1-BOrder-b/view] For this location, a searchable database of materials published since 1988 is available at http://www.pnnl.gov/publications/.

(ix) Provide a list of publicly-available papers and reports resulting from the work during the previous 12 months. (To include authors, titles, and full references.):

1. Hutchison JR, Brooks SM, Kennedy ZC, et al. Polysaccharide-based liquid storage and transport media for non-refrigerated preservation of bacterial pathogens. PLoS One. 2019 Sept 6; 14(9): e0221831. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0221831
2. Hutchison JR, Widder MW, Brooks SM, et al. Consistent Production of Chlorine-Stressed Bacteria from Non-Chlorinated Secondary Sewage Effluents for use in the US Environmental Protection Agency Alternate Test Procedure Protocol. Journal of Microbiological Methods. 2019 June 7; 163 (2019) 105651. https://www.sciencedirect.com/science/article/pii/S016770121930377X?via%3Dihub

3. Cosimbescu L, Malhotra D, Campbell KB, Swita MS, and Kennedy ZC. Molecular Design and Shear Stability Correlations of Dendritic Polymethacrylates. Molecular Systems Design and Engineering. 2019 Oct 2; 4, 1114-1124. https://pubs.rsc.org/en/content/articlehtml/2019/me/c9me00081j
4. Merkley ED, Burnum-Johnson KE, Anderson LN, Jenson SC, and KL Wahl. Uniformly 15N-Labeled Recombinant Ricin A-chain as an Internal Retention Time Standard for Increased Confidence in Forensic Identification of Ricin by Untargeted Nanoflow Liquid Chromatography-Tandem Mass Spectrometry. Analytical Chemistry. 2019 Oct 9; 91, 13372-13376. https://pubs.acs.org/doi/full/10.1021/acs.analchem.9b03389#
5. O'Bryon I, Tucker AE, Kaiser B, Wahl KL, and Merkley ED. Constructing a Tandem Mass Spectral Library for Forensic Ricin Identification. Journal of Proteome Research. 2019 Sept 30; 18, 3926-3935. https://pubs.acs.org/doi/pdf/10.1021/acs.jproteome.9b00377
6. Heller NC, Garrett AM, Merkley ED, et al. Probabilistic Limit of Detection for Ricin Identification Using a Shotgun Proteomics Assay. Analytical Chemistry. 2019 Sept 6; 91, 12399-12406. https://pubs.acs.org/doi/full/10.1021/acs.analchem.9b02721
7. McClure RS, Wendler JP, Adkins JN, et al. Waters KM, and McDermott JE. Unified Feature Association Networks through Integration of Transcriptomic and Proteomic Data. PLoS Computational Biology. 2019 Sept 17; no 15 9:e1007241. https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1007241
8. McDermott JE, Cort JR, Nakayasu ES, et al. Prediction of Bacterial E3 Ubiquitin Ligase Effectors using Reduced Amino Acid Peptide Fingerprinting. PeerJ. 2019 June 7. https://peerj.com/preprints/27292/
9. Couvillion SP, Zhu Y, Nagy G, et al. New mass spectrometry technologies contributing towards comprehensive and high throughput omics analyses of single cells. Analyst. 2019 January 28; 144 no 3:794-807. https://www.ncbi.nlm.nih.gov/pubmed/30507980
10. Kyle JE, Burnum-Johnson KE, Wendler JP, et al. Plasma lipidome reveals critical illness and recovery from human Ebola virus disease. Proceedings of the National Academy of Sciences of the United States of America. 2019 February 26; 116, no 9:3919-3928. https://www.pnas.org/content/116/9/3919
11. Mitchell HD, Eisfeld AJ, Stratton KG, et al. The Role of EGFR in Influenza Pathogenicity: Multiple Network-based Approaches to Identify a Key Regulator of Non-Lethal Infections. Frontiers in Cell and Developmental Biology. 2019 September 20; https://www.frontiersin.org/articles/10.3389/fcell.2019.00200/full
12. Burnum-Johnson KE, Zheng X, Dodds JN, et al. Ion mobility spectrometry and the omics: Distinguishing isomers, molecular classes and contaminant ions in complex samples. Trends in Analytical Chemistry. 2019 July 1; TrAC 116 (2019) 292-299. https://www.sciencedirect.com/science/article/pii/S0165993619300056

5. Briefly describe the biological defence work carried out at the facility, including type(s) of microorganisms* and/or toxins studied, as well as outdoor studies of biological aerosols:

***Objectives*:** PNNL is involved in biodefense-related activities including agent characterization (e.g., knock out experiments and investigation of infectious properties of agents) and the development of detection methods (e.g., nucleic acid, toxin, and proteomic signatures); testing and evaluation of commercial off the shelf equipment for agent detection as well as investigation of next generation biodetection equipment; biological and chemical forensics; investigation of natural history of agents; pathogenesis studies; and interrogating DNA sequencing data and related analysis tools. No outdoor studies of biological aerosols were conducted.

* Including viruses and prions.

***Microorganisms and/or toxins studied*:** Simulants, HHS Select Toxins.

***Outdoor Studies*:** None

Form A, Part 2 (iii)

National biological defence research and development programmes: Facilities

1. What is the name of the facility?
Sandia National Laboratories (SNL)

2. Where is it located (provide both address and geographical location)?
Personnel and budget were shared between two SNL campuses in 2019:

New Mexico Campus: P. O. Box 5800, Albuquerque, NM 87185
(Located on Kirtland Air Force Base, in southeastern Albuquerque)

California Campus: 7011 East Avenue, Livermore, California
(Located in Livermore, CA.)

3. Floor area of laboratory areas by containment level (m^2):

New Mexico campus:	
BSL-2:	1152.45 m^2
BSL-3:	0 m^2
BSL-4:	0 m^2
Total laboratory floor area:	1152.45 m^2
California campus:	
BSL-2:	230 m^2
BSL-3:	0 m^2
BSL-4:	0 m^2
Total laboratory floor area:	230 m^2

Note: Due to changes in project scope in 2019, the BSL-2 laboratory space used for biodefense research and development was decreased from 1268.49 to 1152.45 m^2. The BSL-2 laboratory space was not physically remodeled.

4. The organizational structure of each facility:

(i) Total number of personnel:

New Mexico campus:	256
California campus:	101

(ii) Division of personnel:

Military	0
Civilian	357

(iii) Division of personnel by category:

Scientists	107
Engineers	81
Technicians	145
Admin and Support Staff	24

(iv) List the scientific disciplines represented in the scientific/engineering staff:

Aerobiology, Aerosol Science, Analytical Biochemistry, Analytical Chemistry, Analytical Mass Spectrometry, Bacteriology, Biochemistry, Bioinformatics, Bioinorganic Chemistry, Biological Science, Biomedical Engineering, Biomedical Science, Biophysics, Biotechnology, Cell Biology, Chemical Engineering, Chemistry, Computational Biology, Computer Engineering, Computer Science, Electrical Engineering, Environmental Engineering, Environmental Science, Genetics, Genomics, Immunology, Mass Spectrometry, Materials Science, Mathematics, Mechanical Engineering, Medicine, Microbial Forensics, Microbiology, Molecular Biology, Molecular Diagnostics, Nanotechnology, Neuroscience, Operations Research Analysis, Optical Spectroscopy, Pathology, Physics, Physiology, Polymer Science, Protein Engineering, Proteomics, Structural Biology, Toxicology, Virology.

(v) Are Contractor staff working in the facility?
No

(vi) What is (are) the source(s) of funding for the work conducted in the facility?
U.S. Department of Defense (DoD) - partially
U.S. Department of Health and Human Services (HHS)
Internal (Laboratory Directed Research & Development)
Academia
Private sector

(vii) What are the funding levels for Research and Development and Testing and Evaluation as of the most recent calendar year?

Research	$ 9,164,000
Development	$ 4,131,000
Test and Evaluation	$ 30,000
Total	$ 13,325,000

(viii) Briefly describe the publication policy of the facility:
As a Department of Energy (DOE) facility, Sandia National Laboratories (SNL) is required to make scientific and technical information broadly available, within applicable laws and Departmental requirements, to accomplish mission objectives and strategic goals, promote scientific advancement, satisfy statutory dissemination requirements, and ensure a fair return on Departmental and taxpayer investment. SNL has a mandate to ensure that scientific and technical information is identified, processed, disseminated, and preserved to enable the scientific community and the public to locate and use the unclassified and unlimited-distribution information resulting from DOE research and related endeavors. SNL also has procedures in place to manage and protect classified, sensitive controlled unclassified, and export-controlled scientific and technical information, yet make it accessible for appropriate access by the Department, its contractors, and others. Reviews are conducted prior to publication to determine availability of information, or restrictions thereto. These reviews include, but are not limited to, the following: 1) classification/declassification, 2) copyrighted materials or other intellectual property, 3) export controls or distribution restrictions, and 4) sensitive content that limits access. [Department of Energy, Scientific and Technical Information Management: https://www.directives.doe.gov/directives/0241.1-BOrder-b/view]

(ix) Provide a list of publicly available papers and reports resulting from work during the previous 12 months:

1. Arévalo, M. T., Rizzo, G. M., Polsky, R., et al. Proteomic Characterization of Immunoglobulin Content in Dermal Interstitial Fluid. Journal of Proteome Research. 2019. 10.1021/acs.jproteome.9b00155. https://www.scopus.com/inward/record.uri?eid=2-s2.0-

85066951489&doi=10.1021%2facs.jproteome.9b00155&partnerID=40&md5=dea03d317b72a65780dc3a2ff549302a

2. Caratenuto, R. A., III, Ciabattoni, G. O., DesGranges, et al. Genome sequences of six cluster n mycobacteriophages, kevin1, nenae, parmesanjohn, shrimpfriedegg, smurph, and spongebob, isolated on *Mycobacterium smegmatis* mc2155. Microbiology Resource Announcements. 2019. 10.1128/MRA.00399-19. https://www.scopus.com/inward/record.uri?eid=2-s2.0-85067256661&doi=10.1128%2fMRA.00399-19&partnerID=40&md5=a97c0c4cfabf96a819783bd831e71237
3. Caswell, J., Gans, J. D., Generous, N., Hudson, et al. Defending Our Public Biological Databases as a Global Critical Infrastructure. Frontiers in Bioengineering and Biotechnology. 2019. 10.3389/fbioe.2019.00058. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6460893/
4. Chen, Y., Guenther, J. M., Gin, J. W., et al. Automated "cells-To-Peptides" Sample Preparation Workflow for High-Throughput, Quantitative Proteomic Assays of Microbes. Journal of Proteome Research. 2019. 10.1021/acs.jproteome.9b00455. https://www.scopus.com/inward/record.uri?eid=2-s2.0-85072633124&doi=10.1021%2facs.jproteome.9b00455&partnerID=40&md5=c313858df5766a188c2b2b7603223b6c
5. Jacquet, R., LaBauve, A. E., Akoolo, L., et al. Dual gene expression analysis identifies factors associated with *Staphylococcus aureus* virulence in diabetic mice. Infection and Immunity. 2019. 10.1128/IAI.00163-19. https://www.scopus.com/inward/record.uri?eid=2-s2.0-85065047169&doi=10.1128%2fIAI.00163-19&partnerID=40&md5=2609cd53c4b1c79aefdbefd7d9d301f5
6. Johnston, R. K., Seamon, K. J., Saada, E. A., et al. Use of anti-CRISPR protein AcrIIA4 as a capture ligand for CRISPR/Cas9 detection. Biosensors and Bioelectronics. 2019. 10.1016/j.bios.2019.111361. https://www.scopus.com/inward/record.uri?eid=2-s2.0-85067174275&doi=10.1016%2fj.bios.2019.111361&partnerID=40&md5=2e9d1cac8c399700edc894cd5d4c857d
7. Kinahan, Sean M., Tezak, Matthew S., Siegrist, Cathryn M., et al. Changes of fluorescence spectra and viability from aging aerosolized *E. coli* cells under various laboratory-controlled conditions in an advanced rotating drum. Aerosol Science and Technology. 2019. 10.1080/02786826.2019.1653446. http://dx.doi.org/10.1080/02786826.2019.1653446
8. Lacey, Randy F., Ye, Dongmei and Ruffing, Anne M. Engineering and characterization of copper and gold sensors in *Escherichia coli* and Synechococcus sp. PCC 7002. Applied Microbiology and Biotechnology. 2019. 10.1007/s00253-018-9490-7. http://dx.doi.org/10.1007/s00253-018-9490-7
9. Leynaud-Kieffer, L. M. C., Curran, S. C., Kim, I., et al. A new approach to Cas9-based genome editing in *Aspergillus niger* that is precise, efficient and selectable. PLoS ONE. 2019. 10.1371/journal.pone.0210243. https://www.scopus.com/inward/record.uri?eid=2-s2.0-85060164730&doi=10.1371%2fjournal.pone.0210243&partnerID=40&md5=500f11ab47ebc345c349fbe314e62854
10. Olden, B. R., Perez, C. R., Wilson, A. L., et al. Cell-Templated Silica Microparticles with Supported Lipid Bilayers as Artificial Antigen-Presenting Cells for T Cell Activation. Advanced Healthcare Materials. 2019. 10.1002/adhm.201801188. https://onlinelibrary.wiley.com/doi/full/10.1002/adhm.201801188
11. Phanouf, Christopher R., Seamon, Kyle J., Eckles, Tyler P., et al. Ultrasensitive multi-species detection of CRISPR-Cas9 by a portable centrifugal microfluidic platform. Analytical Methods. 2019. 10.1039/c8ay02726a. http://dx.doi.org/10.1039/c8ay02726a
12. Sava Gallis, Dorina F., Butler, Kimberly S., Agola, Jacob O., et al. Antibacterial Countermeasures via Metal-Organic Framework-Supported Sustained Therapeutic Release Journal: ACS Applied Materials and Interfaces. 2019. 10.1021/acsami.8b21698. http://dx.doi.org/10.1021/acsami.8b21698

5. Briefly describe the biological defense work carried out at the facility, including type(s) of microorganisms* and/or toxins studied, as well as outdoor studies of biological aerosols.

Objectives: To improve the United States' ability to anticipate and defend against biological threats, SNL's multidisciplinary research team applies its traditional strengths in engineering and technology development to achieve the following goals: 1) gain basic knowledge regarding the fundamental molecular processes of pathogenesis, including the dynamic interactions between microbial pathogens and their hosts; 2) develop assays, novel materials, and platforms to detect and diagnose traditional and unknown pathogens, as well as to discover novel therapeutic targets; and 3) obtain an understanding of the microbiome's effects on human health in the absence or in the presence of an infectious disease.

***Microorganisms and/or toxins studied*:** None

***Outdoor studies*:** None

* Including viruses and prions.

Form A, Part 2 (iii)

National biological defence research and development programmes

1. What is the name of the facility?
Centers for Disease Control and Prevention (CDC), National Center for Environmental Health (NCEH), Division of Laboratory Services (DLS)

2. Where is it located (include both address and geographical location)?
4770 Buford Highway, Atlanta, Georgia 30341

3. Floor area of laboratory areas by containment level (m^2):

BSL-2	568 m^2
BSL-3	0 m^2
BSL-4	0m^2
Total laboratory floor area	568 m^2

4. The organizational structure of each facility.

(i) Total number of personnel 18

(ii) Division of personnel:

Military	0
Civilian	18

(iii) Division of personnel by category:

Scientists	18
Engineers	0
Technicians	0
Administrative and support staff	0

(iv) List the scientific disciplines represented in the scientific/engineering staff.
Analytical Biochemistry, Analytical Chemistry, Analytical Mass Spectrometry, Biochemistry, Biology, Chemistry, Mass Spectrometry, Proteomics

(v) Are contractor staff working in the facility? If so, provide an approximate number.
Yes Number: 5

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
Centers for Disease Control and Prevention, Department of Health and Human Services (HHS)

(vii) What are the funding levels for the following programme areas:

Research	$2,034,629.73
Development	$998,180.00
Test and evaluation	$2,215,920.05
Total	$5,248,729.78

(viii) Briefly describe the publication policy of the facility:
Scientists are encouraged to publish their results in the peer reviewed scientific literature as well as present their work at national and international professional meetings. The clearance

policy for information products disseminated outside CDC for public use is available online at: http://www.cdc.gov/od/science/policies. CDC also has an internal policy on "Oversight and clearance of dual use research of concern."

(ix) Provide a list of publicly-available papers and reports resulting from the work published during the previous 12 months. (To include authors, titles and full references.)

1. Horowitz BZ, Castelli R, Hughes A, Hendrickson RG, Johnson RC, Thomas JD. Massive fatal overdose of abrin with progressive encephalopathy [published online ahead of print, 2019 Aug 28]. Clin Toxicol (Phila). 2019;1–4. doi:10.1080/15563650.2019.1655150 https://www.tandfonline.com/doi/full/10.1080/15563650.2019.1655150
2. Kalb SR, Baudys J, Kiernan K, Wang D, Becher F, and Barr JR. Proposed BoNT/A and /B Peptide Substrates Cannot Detect Multiple Subtypes in the Endopep-MS Assay [published online ahead of print, 2019 Jul 9]. J Anal Toxicol. https://academic.oup.com/jat/advance-article-abstract/doi/10.1093/jat/bkz044/5530128?redirectedFrom=fulltext
3. Lins RC, Boyer AE, Kuklenyik Z, Woolfitt AR, Goldstein J, Hoffmaster AR, et al. Zeptomole per milliliter detection and quantification of edema factor in plasma by LC-MS/MS yields insights into toxemia and the progression of inhalation anthrax. Anal. Bioanal. Chem. 2019, May; 411(12):2493-2509. https://link.springer.com/article/10.1007%2Fs00216-019-01730-4
4. Solano MI, Woolfitt AR, Boyer AE, Lins RC, Isbell K, Gallegos-Candela M et al. Accurate and selective quantification of anthrax protective antigen in plasma by immunocapture and isotope dilution mass spectrometry. Analyst, 2019, Mar 25; 144(7):2264-2274. https://pubs.rsc.org/en/content/articlelanding/2019/AN/C8AN02479K#!divAbstract
5. Wang D, Baudys J, Hoyt K, Barr JR, and Kalb SR. Sensitive Detection of Type G Botulinum Neurotoxin through Endopep-MS Peptide Substrate Optimization. Anal. Bioanal.Chem. 2019 Aug; 411(21): 5489-5497. https://link.springer.com/article/10.1007%2Fs00216-019-01926-8
6. Wharton RE, Cunningham BR, Schaefer AM, Guldberg SM, Hamelin EI, Johnson RC. Measurement of Microcystin and Nodularin Activity in Human Urine by Immunocapture-Protein Phosphatase 2A Assay. Toxins (Basel). 2019 Dec 13; 11(12):729. doi:10.3390/toxins11120729 https://www.scilit.net/article/4a9eca0b1a8fc150a34d676fa695d013?action=show-references

5. Briefly describe the biological defence work carried out at the facility, including type(s) of micro-organisms* and/or toxins studied, as well as outdoor studies of biological aerosols.

Objectives: The Division of Laboratory Sciences develops methods for measuring selected toxins to help improve detection and diagnosis during a public health response to biological toxins.

Agents Microorganisms and/or toxins studied: Select Agents (HHS), Select Toxins (HHS), NIAID Category A Pathogens

Outdoor studies: Outdoor studies of biological aerosols were not conducted at the facility or off-site by facility personnel.

* Including viruses and prions.

Form A, Part 2 (iii)

National biological defence research and development programmes

1. What is the name of the facility?
Centers for Disease Control and Prevention (CDC), Deputy Director for Infectious Diseases (DDID)

2. Where is it located (include both address and geographical location)?
1600 Clifton Road N.E., Atlanta, Georgia 30329

3. Floor area of laboratory areas by containment level (m^2):

BSL-2	423 m^2
BSL-3	1220 m^2
BSL-4	533 m^2
Total laboratory floor area	2176 m^2

4. The organizational structure of each facility.

(ii) Total number of personnel: 222

(ii) Division of personnel:

Military	6
Civilian	216

(iii) Division of personnel by category:

Scientists	186
Engineers	0
Technicians	17
Administrative and support staff	19

(iv) List the scientific disciplines represented in the scientific/engineering staff.
Animal Science, Biochemistry, Bioinformatics, Biology, Biological Science, Cell Biology, Chemistry, Clinical Immunology, Ecology, Entomology, Epidemiology, Genetics, Genomics, Immunology, Medicine, Microbiology, Molecular Biology, Molecular Diagnostics, Public Health, Statistics, Veterinary Medicine, Virology

(v) Are contractor staff working in the facility? If so, provide an approximate number.
Yes Number: 45

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
U.S. Department of Health and Human Services (HHS)
U.S. Department of Homeland Security (DHS)
U.S. Department of Defense
U.S. Agency for International Development (USAID)

(vii) What are the funding levels for the following programme areas:

Research	$14,205,315
Development	$5,785,469
Test and evaluation	$8,495,700
Total	$28,486,484

(viii) Briefly describe the publication policy of the facility:
Publication is encouraged and managed by editorial and clearance policies conducted at all levels of the Agency. The clearance policy for information products disseminated outside CDC for public use is available online at: http://www.cdc.gov/od/science/policies. CDC also has an internal policy on "Oversight and clearance of dual use research of concern."

(ix) Provide a list of publicly-available papers and reports resulting from the work published during the previous 12 months. (To include authors, titles and full references.)

1. Ganapathiraju PV, Gharpure R, Thomas D, Millet N, Gurrieri D, Chatham Stephens K et al. Notes from the Field: Botulism Type E After Consumption of Salt-Cured Fish - New Jersey, 2018. MMWR. 2019 Nov 8; 68(44):1008-1009. doi: 10.15585/mmwr.mm6844a3. https://www.ncbi.nlm.nih.gov/pubmed/31697653
2. Halpin JL, Dykes JK, Katz L, Centurioni DA, Perry MJ, Egan CT, Lúquez C. Molecular Characterization of Clostridium botulinum Harboring the bont/B7 Gene. Foodborne Pathog Dis. 2019 Jun; 16(6):428-433. doi: 10.1089/fpd.2018.2600. Epub 2019 Mar 30. https://www.ncbi.nlm.nih.gov/pubmed/30932710
3. Bergeron G, Latash J, Da Costa-Carter CA, Egan C, Stavinsky F, Kileci JA et al. Notes from the Field: Botulism Outbreak Associated with Home-Canned Peas - New York City, 2018. MMWR. 2019 Mar 15; 68(10):251-252. doi: 10.15585/mmwr.mm6810a5. https://www.ncbi.nlm.nih.gov/pubmed/30870408
4. Halpin JL, Wangroongsarb P, Jittaprasartsin C, Dykes JK, Lúquez C. Draft Genome Sequence of a Clostridium botulinum Isolate from Thailand Harboring the Subtype bont/B8 Gene. Microbiol Resour Announc. 2019 Jan 31; 8(5). pii: e01216-18. doi: 10.1128/MRA.01216-18. https://www.ncbi.nlm.nih.gov/pubmed/30714030
5. Halpin JL, Dykes JK, Lúquez C. Draft Genome Sequences for Dual-Toxin-Producing Clostridium botulinum Strains. Microbiol Resour Announc. 2019 Jan 24; 8(4). pii: e01152-18. doi: 10.1128/MRA.01152-18.https://www.ncbi.nlm.nih.gov/pubmed/30701230
6. Whitehouse ER. Novel treatment of a Vaccinia virus infection from an occupational needlestick San Diego, California, 2019. MMWR. 2019; 68:943-6. https://www.cdc.gov/mmwr/volumes/68/wr/mm6842a2.htm?s_cid=mm6842a2_w
7. Weiner ZP, Salzer JS, LeMasters E, et al. Characterization of Monkeypox virus dissemination in the black-tailed prairie dog (Cynomys ludovicianus) through in vivo bioluminescent imaging. PloS One 2019; 14:e0222612. https://doi.org/10.1371/journal.pone. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6762066/
8. Reynolds MG, Wauquier N, Li Y, et al. Human Monkeypox in Sierra Leone after 44-Year Absence of Reported Cases. Emerging Inf Dis. 2019; 25:1023-5. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6478203/
9. Reynolds MG, Doty JB, McCollum AM, Olson VA, Nakazawa Y. Re-emergence of Monkeypox in Africa: a Call to Expand the Concept and Practice of One Health. Expert Reviews in Anti-Infective Therapy. 2019; 17:129-39. DOI: 10.1080/14787210.2019.1567330. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6438170/
10. Merchlinsky M, Albright A, Olson V, et al. The development and approval of tecovirimat (TPOXX®), the first antiviral against smallpox. Antiviral Res. 2019; 168:168-74. https://www.ncbi.nlm.nih.gov/pubmed/?term=The+development+and+approval+of+tecovirimat+(TPOXX%C2%AE)%2C+the+first+antiviral+against+smallpox
11. Lindholm DA, Fisher RD, Montgomery JR, et al. Preemptive tecovirimat use in an active duty member presenting with acute myeloid leukemia after smallpox vaccination. Clin Inf Dis. 2019; 69:2205-7. DOI: 10.1093/cid/ciz286.

https://www.ncbi.nlm.nih.gov/pubmed/?term=Preemptive+tecovirimat+use+in+an+active+duty+member

12. Leung J, McCollum AM, Radford K, et al. Varicella in Tshuapa Province, Democratic Republic of the Congo, 2009-2014. Trop Med Int Health 2019; 24:839-48. doi:10.1111/tmi.13243. https://www.ncbi.nlm.nih.gov/pubmed/?term=Varicella+in+Tshuapa+Province%2C+Democratic+Republic+of+the+Congo%2C+2009-2014
13. Jeon S, Cleaton J, Meltzer MI, et al. Determining the post-elimination level of vaccination needed to prevent re-establishment of dog rabies. PLoS Neglected Tropical Diseases. 2019; 13:e0007869. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6907870/
14. Hutson CL, Gallardo-Romero N, Carroll DS, et al. Analgesia during Experimental Challenge Studies of Monkeypox Virus in Prairie Dogs (Cynomys ludovicianus). J Am Assoc Lab Anim Sci. 2019; 58:1-16. DOI: 0.30802/AALAS-JAALAS-18-000036. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6643093/
15. Guagliardo SAJ, Monroe B, Moundjoa C, et al. Asymptomatic Orthopoxvirus circulation in humans in the wake of a monkeypox outbreak among chimpanzees in Cameroon. AM J trop Med Hyg. 2019: DOI: https://doi.org/10.4269/ajtmh.19-0467 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6947779/
16. Guagliardo SAJ, Doshi RH, Reynolds MG, et al. Do monkeypox exposures vary by ethnicity? Comparison of Aka- and Bantu-suspected monkeypox cases. AM J Trop Med Hyg. 2019: doi:10.4269/ajtmh.19-0457. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6947767/
17. Gigante CM, Gao J, Tang S, et al. Genome of Alaskapox virus, a novel orthopoxvirus isolated from Alaska. Viruses. 2019; 11:708; https://doi.org/10.3390/v11080708. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6723315/
18. Doty JB, Maghlakelidze G, Sikharulidze I, et al. Isolation and characterization of Akhmeta virus from wild-caught rodents (Apodemus spp.) in Georgia. J Virol. 2019; 93:e00966-19. doi: 10.1128/JVI.-19 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6880181/
19. Doshi RH, Guagliardo SAJ, Doty JB, et al. Epidemiologic and Ecologic Investigations of Monkeypox, Likouala Department, Republic of the Congo, 2017. Emerg Infect Dis. 2019; 25:281-9. https://wwwnc.cdc.gov/eid/article/25/2/18-1222_article
20. Angelo KM, Petersen BW, Hamer DH, Schwartz E, Brunette G. Monkeypox transmission among international travellers – serious monkey business? J Travel Med. 2019;1-2. doi: 10.1093/jtm/taz002. https://www.ncbi.nlm.nih.gov/pubmed/?term=Monkeypox+transmission+among+international+travellers+serious+monkey+business
21. Donahue MA, Newcomb G, Spinella S, et al. CNS Melioidosis in a Traveler Returning from Cabo, Mexico. Open Forum Infect Dis. 2019; 6(2):ofz005. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6377934/
22. Hall CM, Jaramillo S, Jimenez R, et al. Burkholderia pseudomallei, the causative agent of melioidosis, is rare but ecologically established and widely dispersed in the environment in Puerto Rico. PLoS Neglected Tropical Diseases. 2019; 13(9):e0007727. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6748447/
23. Nolen LD, Lirow E, Gee JE, Elrod MG, Kolton CB, Liu L et al. Differentiating New from Newly Detected: Melioidosis in Yap, Federated States of Micronesia. Am J Trop Med Hyg. 2019; 101(2):323-327. http://www.ajtmh.org/docserver/fulltext/14761645/101/2/tpmd190253.pdf?expires=1580318212&id=id&accname=11600&checksum=1B9ABCAE8CE1A1A1CCE7D6695A073E80
24. Kolton CB, Marston CK, Stoddard RA, et al. Detection of Bacillus anthracis in animal tissues using InBios active anthrax detect rapid test lateral flow immunoassay. Lett Appl Microbiol. 2019. https://sfamjournals.onlinelibrary.wiley.com/doi/full/10.1111/lam.13134
25. Pillai SP, Prentice KW, Ramage JG, et al. Rapid Presumptive Identification of Bacillus anthracis Isolates Using the Tetracore RedLine Alert Test. Health Security. 2019; 17(4):334-343.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6708271/
26. Cossaboom CM, Khaiseb S, Haufiku B, et al. Anthrax Epizootic in Wildlife, Bwabwata National Park, Namibia, 2017. Emerg Infect Dis. 2019; 25(5):947-950. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6478215/
27. Gargis AS, Lascols C, McLaughlin HP, Conley AB, Hoffmaster AR, Sue D. Genome Sequences of Penicillin-Resistant Bacillus anthracis Strains. Microbiol Resour Announc. 2019; 8(2). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6328647/
28. Guendel I, Ekpo LL, Hinkle MK, et al. Melioidosis after Hurricanes Irma and Maria, St. Thomas/St. John District, US Virgin Islands, October 2017. Emerg Infect Dis. 2019; 25(10):1952-1955. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6759263/
29. Abudurexiti A, Adkins S, Alioto D, et al. Taxonomy of the order Bunyavirales: update 2019. Arch Virol. 2019;164(7):1949-1965. https://www.ncbi.nlm.nih.gov/pubmed/31065850
24. Amarasinghe GK, Ayllon MA, Bao Y, et al. Taxonomy of the order Mononegavirales: update 2019. Arch Virol. 2019; 164(7):1967-1980. https://www.ncbi.nlm.nih.gov/pubmed/31089958
25. Bruhn JF, Hotard AL, Spiropoulou CF, Lo MK, Saphire EO. A Conserved Basic Patch and Central Kink in the Nipah Virus Phosphoprotein Multimerization Domain Are Essential for Polymerase Function. Structure (London, England: 1993). 2019; 27(4):660-668.e664. https://www.ncbi.nlm.nih.gov/pubmed/?term=Conserved+Basic+Patch+and+Central+Kink+in+the+Nipah+Virus+Phosphoprotein+Multimerization+Domain+Are+Essential+for+Polymerase+Function
26. Davis CW, Jackson KJL, McElroy AK, et al. Longitudinal Analysis of the Human B Cell Response to Ebola Virus Infection. Cell. 2019;177(6):1566-1582.e1517. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6908968/
27. Dzimianski JV, Scholte FEM, Williams IL, et al. Determining the molecular drivers of species-specific interferon-stimulated gene product 15 interactions with nairovirus ovarian tumor domain proteases. PloS One. 2019;14(12): e0226415. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6927636/
28. Fagre AC, Lee JS, Kityo RM, et al. Discovery and Characterization of Bukakata orbivirus (Reoviridae: Orbivirus), a Novel Virus from a Ugandan Bat. Viruses. 2019; 11(3). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6466370/
29. Fernando R, Capone D, Elrich S, et al. Infection with New York Orthohantavirus and Associated Respiratory Failure and Multiple Cerebral Complications. Emerg Infect Dis. 2019; 25(6):1241-1243. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6537728/
30. Flint M, Chatterjee P, Lin DL, et al. A genome-wide CRISPR screen identifies N-acetylglucosamine-1-phosphate transferase as a potential antiviral target for Ebola virus. Nat Commun. 2019; 10(1):285. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6336797/
31. Gary JM, Welch SR, Ritter JM, et al. Lassa Virus Targeting of Anterior Uvea and Endothelium of Cornea and Conjunctiva in Eye of Guinea Pig Model. Emerg Infect Dis. 2019; 25(5):865-874. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6478213/
32. Genzer SC, Huynh T, Coleman-Mccray JD, Harmon JR, Welch SR, Spengler JR. Hematology and Clinical Chemistry Reference Intervals for Inbred Strain 13/n Guinea Pigs (Cavia Porcellus). J Am Assoc Lab Anim Sci. 2019; 58(3):293-303. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6526494/
33. Genzer SC, Welch SR, Scholte FEM, et al. Alterations in Blood Chemistry Levels Associated With Nipah Virus Disease in the Syrian Hamster Model. J Infect Dis. 2019. https://www.ncbi.nlm.nih.gov/pubmed/?term=Alterations+in+Blood+Chemistry+Levels+Associated+With+Nipah+Virus+Disease+in+the+Syrian+Hamster+Model
34. He YX, Ye CL, Zhang P, et al. Yersinia pseudotuberculosis Exploits CD209 Receptors for Promoting Host Dissemination and Infection. Infect Immun. 2019; 87(1). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6300620/
35. Hegde ST, Salje H, Sazzad HMS, et al. Using healthcare-seeking behaviour to estimate the number of

Nipah outbreaks missed by hospital-based surveillance in Bangladesh. Int J Epidemiol. 2019; 48(4):1219-1227. https://www.ncbi.nlm.nih.gov/pubmed/?term=Using+healthcare-seeking+behaviour+to+estimate+the+number+of+Nipah+outbreaks+missed+by+hospital-based+surveillance+in+Bangladesh

36. Jones MEB, Amman BR, Sealy TK, et al. Clinical, Histopathologic, and Immunohistochemical Characterization of Experimental Marburg Virus Infection in A Natural Reservoir Host, the Egyptian Rousette Bat (Rousettus aegyptiacus). Viruses. 2019; 11(3). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6466277/
37. Kainulainen MH, Spengler JR, Welch SR, et al. Protection From Lethal Lassa Disease Can Be Achieved Both Before and After Virus Exposure by Administration of Single-Cycle Replicating Lassa Virus Replicon Particles. J Infect Dis. 2019; 220(8):1281-1289. https://www.ncbi.nlm.nih.gov/pubmed/?term=Protection+From+Lethal+Lassa+Disease+Can+Be+Achieved+Both+Before+and+After+Virus+Exposure+by+Administration+of+Single-Cycle+Replicating+Lassa+Virus+Replicon+Particles
38. Kuhn JH, Amarasinghe GK, Basler CF, et al. ICTV Virus Taxonomy Profile: Filoviridae. J Gen Virol. 2019;100(6):911-912. https://www.microbiologyresearch.org/content/journal/jgv/10.1099/jgv.0.001252#tab2
39. Liu WJ, Sesay FR, Coursier A, et al. Comprehensive Clinical and Laboratory Follow-up of a Female Patient With Ebola Virus Disease: Sierra Leone Ebola Virus Persistence Study. Open Forum Infect Dis. 2019;6(3): ofz068. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6440679/
40. Lo MK, Feldmann F, Gary JM, et al. Remdesivir (GS-5734) protects African green monkeys from Nipah virus challenge. Sci Transl Med. 2019; 11(494). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6732787/
41. Lo MK, Spengler JR, Welch SR, et al. Evaluation of a Single-Dose Nucleoside-Modified Messenger RNA Vaccine Encoding Hendra Virus-Soluble Glycoprotein Against Lethal Nipah virus Challenge in Syrian Hamsters. J Infect Dis. 2019. https://www.ncbi.nlm.nih.gov/pubmed/?term=Evaluation+of+a+Single-Dose+Nucleoside-Modified+Messenger+RNA+Vaccine+Encoding+Hendra+Virus-Soluble+Glycoprotein+Against+Lethal+Nipah+virus+Challenge+in+Syrian+Hamsters.
42. Maes P, Amarasinghe GK, Ayllon MA, et al. Taxonomy of the order Mononegavirales: second update 2018. Arch Virol. 2019; 164(4):1233-1244. https://www.ncbi.nlm.nih.gov/pubmed/?term=Taxonomy+of+the+order+Mononegavirales%3A+second+update+2018
43. McElroy AK, Shrivastava-Ranjan P, Harmon JR, et al. Macrophage Activation Marker Soluble CD163 Associated with Fatal and Severe Ebola Virus Disease in Humans(1). Emerg Infect Dis. 2019; 25(2):290-298. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6346465/
44. McMullan LK, Flint M, Chakrabarti A, et al. Characterisation of infectious Ebola virus from the ongoing outbreak to guide response activities in the Democratic Republic of the Congo: a phylogenetic and in vitro analysis. Lancet Infect Dis. 2019; 19(9):1023-1032. https://www.sciencedirect.com/science/article/pii/S1473309919302919?via%3Dihub
45. Nyakarahuka L, Shoemaker TR, Balinandi S, et al. Marburg virus disease outbreak in Kween District Uganda, 2017: Epidemiological and laboratory findings. *PLoS Neglected Trop Dis.* 2019;13(3): e0007257. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6438581/
46. Pickering BS, Spengler JR, Shadabi E, Dalziel AE, Lautner EA, Silva P. The Biosafety Level 4 Zoonotic Laboratory Network (BSL4ZNet): Report of a workshop on live animal handling. Antiviral Res. 2019; 172:104640. https://www.sciencedirect.com/science/article/pii/S0166354219305522?via%3Dihub
47. Prescott J, Guito JC, Spengler JR, et al. Rousette Bat Dendritic Cells Overcome Marburg Virus-Mediated Antiviral Responses by Upregulation of Interferon-Related Genes While Downregulating Proinflammatory Disease Mediators. mSphere. 2019; 4(6).

48. https://www.ncbi.nlm.nih.gov/pubmed/31801842Reichler MR, Bruden D, Thomas H, et al. Ebola Patient Virus Cycle Threshold and Risk of Household Transmission of Ebola Virus. J Infect Dis. 2019. https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiz511/5681532
49. Scholte FEM, Hua BL, Spengler JR, et al. Stable Occupancy of the Crimean-Congo Hemorrhagic Fever Virus-Encoded Deubiquitinase Blocks Viral Infection. mBio. 2019; 10(4). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6650548/
50. Scholte FEM, Spengler JR, Welch SR, et al. Single-dose replicon particle vaccine provides complete protection against Crimean-Congo hemorrhagic fever virus in mice. Emerg Microbes Infect. 2019;8(1):575-578. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6455139/
51. Schuh AJ, Amman BR, Sealy TK, et al. Antibody-Mediated Virus Neutralization Is Not a Universal Mechanism of Marburg, Ebola, or Sosuga Virus Clearance in Egyptian Rousette Bats. J Infect Dis. 2019; 219(11):1716-1721. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6519049/
52. Schuh AJ, Amman BR, Sealy TS, et al. Comparative analysis of serologic cross-reactivity using convalescent sera from filovirus-experimentally infected fruit bats. Sci Rep. 2019; 9(1):6707. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6491471/
53. Shastri B, Kofman A, Hennenfent A, et al. Domestically Acquired Seoul Virus Causing Hemophagocytic Lymphohistiocytosis-Washington, DC, 2018. Open Forum Infect Dis. 2019; 6(10):ofz404. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6790396/
54. Spengler JR, Bergeron E, Spiropoulou CF. Crimean-Congo hemorrhagic fever and expansion from endemic regions. Curr Opin Virol. 2019;34:70-78. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6497153/
55. Spengler JR, Welch SR, Genzer SC, et al. Suboptimal Handling of Piccolo Samples or Reagent Discs for Consideration in Ebola Response. Emerg Infect Dis. 2019; 25(6):1238-1240. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6537746/
56. Spengler JR, Welch SR, Scholte FEM, et al. Heterologous protection against Crimean-Congo hemorrhagic fever in mice after a single dose of replicon particle vaccine. Antiviral Res. 2019; 170:104573. https://www.ncbi.nlm.nih.gov/pubmed/?term=Heterologous+protection+against+CrimeanCongo+hemorrhagic+fever+in+mice+after+a+single+dose+of+replicon+particle+vaccine
57. Spiropoulou CF. Nipah Virus Outbreaks: Still Small but Extremely Lethal. J Infect Dis. 2019; 219(12):1855-1857. https://academic.oup.com/jid/article/219/12/1855/5144923
58. Tramontano E, Tarbet B, Spengler JR, et al. Meeting report: 32nd International Conference on Antiviral Research. Antiviral Res. 2019; 169:104550. https://www.sciencedirect.com/science/article/pii/S0166354219303596?via%3Dihub
59. Welch SR, Ritter JM, McElroy AK, et al. Fluorescent Crimean-Congo hemorrhagic fever virus illuminates tissue tropism patterns and identifies early mononuclear phagocytic cell targets in IFNAR-/- mice. PLoS Pathogens. 2019; 15(12):e1008183. https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1008183
60. Welch SR, Scholte FEM, Harmon JR, et al. In Situ Imaging of Fluorescent Nipah Virus Respiratory and Neurological Tissue Tropism in the Syrian Hamster Model. J Infect Dis. 2019. https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiz393/5610252
61. Welch SR, Scholte FEM, Harmon JR, et al. Corrigendum to: In Situ Imaging of Fluorescent Nipah Virus Respiratory and Neurological Tissue Tropism in the Syrian Hamster Model. J Infect Dis. 2019. https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiz627/5691358
62. Wiley MR, Fakoli L, Letizia AG, et al. Lassa virus circulating in Liberia: a retrospective genomic characterisation. Lancet Infect Dis. 2019; 19(12):1371-1378. https://www.sciencedirect.com/science/article/pii/S1473309919304864?via%3Dihub
63. Yadav PD, Whitmer SLM, Sarkale P, et al. Characterization of Novel Reoviruses Wad Medani Virus (Orbivirus) and Kundal Virus (Coltivirus) Collected from Hyalomma anatolicum Ticks in India during Surveillance for Crimean Congo Hemorrhagic Fever. J Virol. 2019; 93(13).

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6580951/
64. Yang K, He Y, Park CG, et al. Yersinia pestis Interacts With SIGNR1 (CD209b) for Promoting Host Dissemination and Infection. Front Immunol. 2019; 10:96. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6422942/
65. Yates MK, Chatterjee P, Flint M, et al. Probing the Effects of Pyrimidine Functional Group Switches on Acyclic Fleximer Analogues for Antiviral Activity. Molecules (Basel, Switzerland). 2019; 24(17). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6749450/
66. Ye C, Li Q, Li X, et al. Salmonella enterica Serovar Typhimurium Interacts with CD209 Receptors To Promote Host Dissemination and Infection. Infect Immun. 2019; 87(8). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6652768/
67. Gargis AS, Lascols C, McLaughlin HP, Conley AB, Hoffmaster AR, Sue D. Genome Sequences of Penicillin Resistant Bacillus anthracis Strains. Microbiol Resour Announc. 2019 Jan 108(2). https://mra.asm.org/content/8/2/e01122-18
68. Gargis, A.S., Cherney, B., Conley, A.B. et al. Rapid Detection of Genetic Engineering, Structural Variation, and Antimicrobial Resistance Markers in Bacterial Biothreat Pathogens by Nanopore Sequencing. Sci Rep 9, 13501 (2019). https://www.nature.com/articles/s41598-019-49700-1
69. Villanueva, J., Schweitzer, B., Odle, M., & Aden, T. (2019). Detecting Emerging Infectious Diseases: An Overview of the Laboratory Response Network for Biological Threats. Public Health Rep, 134(2 suppl), 16S-21S. https://journals.sagepub.com/doi/10.1177/0033354919874354
70. Miernyk KM, Bruden D, Parkinson AJ, Hurlburt D, Klejka J, Berner J et al. Human Seroprevalence to 11 Zoonotic Pathogens in the U.S. Arctic, Alaska. Vector Borne Zoonotic Dis. 2019 Aug; 19(8):563-575. doi: 10.1089/vbz.2018.2390. Epub 2019 Feb 21.https://www.ncbi.nlm.nih.gov/pubmed/30789314
71. Wiley Z, Reddy S, Jacobs Slifka KM, Brandon DC, Jernigan J, Kersh GJ, Armstrong PA. Chronic Q Fever with Vascular Involvement: Progressive Abdominal Pain in a Patient with Aortic Aneurysm Repair in the United States. Case Rep Infect Dis. 2019 Feb 19;2019 https://www.ncbi.nlm.nih.gov/pubmed/30915246
72. Kersh GJ, Fitzpatrick K, Pletnikoff K, Brubaker M, Bruce M, Parkinson A. Prevalence of serum antibodies to Coxiella burnetii in Alaska Native Persons from the Pribilof Islands. Zoonoses Public Health. 2020 Feb; 67(1):89-92. doi: 10.1111/zph.12661. Epub 2019 Nov 8; https://www.ncbi.nlm.nih.gov/pubmed/31705592
73. Smith CB, Evavold C, Kersh GJ. The Effect of pH on Antibiotic Efficacy against Coxiella burnetii in Axenic Media. Sci Rep. 2019 Dec 2; 9(1):18132; https://www.ncbi.nlm.nih.gov/pubmed/31792307
74. Andayi F, Chaves SS, Widdowson MA. Impact of the 1918 Influenza Pandemic in Coastal Kenya. Trop Med Infect Dis. 2019 Jun 8; 4(2). pii: E91. doi: 10.3390/tropicalmed4020091, PubMed Central: https://www.ncbi.nlm.nih.gov/pubmed/31181715
75. Jester B, Uyeki TM, Jernigan DB, Tumpey TM. Historical and clinical aspects of the 1918 H1N1 pandemic in the United States. Virology. 2019; 527:32-37. PubMed Central: https://www.ncbi.nlm.nih.gov/pubmed/30453209
76. Lafond KE, Praptiningsih CY, Mangiri A, Syarif M, Triada R, Mulyadi E, et al. Seasonal Influenza and Avian Influenza A(H5N1) Virus Surveillance among Inpatients and Outpatients, East Jakarta, Indonesia, 2011-2014. Emerg Infect Dis. 2019; 25:2031-2039. PubMed Central: https://www.ncbi.nlm.nih.gov/pubmed/31625837
77. Levine MZ, Holiday C, Jefferson S, Gross FL, Liu F, Li S, et al. Heterologous prime-boost with A(H5N1) pandemic influenza vaccines induces broader cross-clade antibody responses than homologous prime-boost. NPJ Vaccines. 2019; 4:22. PubMed Central: https://www.ncbi.nlm.nih.gov/pubmed/31149353
78. Olsen SJ, Rooney JA, Blanton L, Rolfes MA, Nelson DI, Gomez TM at al. Estimating Risk to Responders Exposed to Avian Influenza A H5 and H7 Viruses in Poultry, United States, 2014-2017.

Emerg Infect Dis. 2019; 25:1011-1014. PubMed Central: https://www.ncbi.nlm.nih.gov/pubmed/30741630
79. Poirot E, Levine MZ, Russell K, Stewart RJ, Pompey JM, Chiu S et al. Detection of Avian Influenza A(H7N2) Virus Infection Among Animal Shelter Workers Using a Novel Serological Approach-New York City, 2016-2017. J Infect Dis. 2019; 219:1688-1696. PubMed Central: https://www.ncbi.nlm.nih.gov/pubmed/30395249
80. Sun X, Belser JA, Pappas C, Pulit-Penaloza JA, Brock N, Zeng H et al. Correction for Sun et al., Risk Assessment of Fifth-Wave H7N9 Influenza A Viruses in Mammalian Models. J Virol. 2019 June 14; 93(13). PubMed Central: https://www.ncbi.nlm.nih.gov/pubmed/31201279
81. Sun X, Belser JA, Pappas C, Pulit-Penaloza JA, Brock N, Zeng H, Creager HM et al. Risk Assessment of Fifth-Wave H7N9 Influenza A Viruses in Mammalian Models. J Virol. 2018 Dec 10; 93(1). pii: e01740-18. doi: 10.1128/JVI.01740-18. Print 2019 Jan 1. PubMed Central: https://www.ncbi.nlm.nih.gov/pubmed/30305359
82. Sun X, Belser JA, Yang H, Pulit-Penaloza JA, Pappas C, Brock N et al. Identification of key hemagglutinin residues responsible for cleavage, acid stability, and virulence of fifth-wave highly pathogenic avian influenza A(H7N9) viruses. Virology. 2019; 535:232-240. PubMed Central: https://www.ncbi.nlm.nih.gov/pubmed/31325838
83. Wang X, Wu P, Pei Y, Tsang TK, Gu D, Wang W et al. Assessment of Human-to-Human Transmissibility of Avian Influenza A(H7N9) Virus Across 5 Waves by Analyzing Clusters of Case Patients in Mainland China, 2013-2017. Clin Infect Dis. 2019; 68:623-631. PubMed Central: https://www.ncbi.nlm.nih.gov/pubmed/29961834
84. Yang G, Chowdury S, Hodges E, Rahman MZ, Jang Y, Hossain ME et al. Detection of highly pathogenic avian influenza A(H5N6) viruses in waterfowl in Bangladesh. Virology. 2019; 534:36-44. PubMed Central: https://www.ncbi.nlm.nih.gov/pubmed/31176062
85. Zeng H, Goldsmith CS, Kumar A, Belser JA, Sun X, Pappas C et al. Tropism and Infectivity of a Seasonal A(H1N1) and a Highly Pathogenic Avian A(H5N1) Influenza Virus in Primary Differentiated Ferret Nasal Epithelial Cell Cultures. J Virol. 2019 May 1; 93(10). PubMed Central: https://www.ncbi.nlm.nih.gov/pubmed/30814288
86. Zhong W, Levine MZ. Stockpiled Avian Influenza A(H7N9) Vaccines Induce Robust, Nonneutralizing Functional Antibodies Against Antigenically Drifted Fifth-Wave A(H7N9) Viruses. J Infect Dis. 2019; 220:1276-1280. PubMed Central: https://www.ncbi.nlm.nih.gov/pubmed/31169293
87. Zhou L, Li Q, Uyeki TM. Estimated Incubation Period and Serial Interval for Human-to-Human Influenza A(H7N9) Virus Transmission. Emerg Infect Dis. 2019; 25:1982-1983. PubMed Central: https://www.ncbi.nlm.nih.gov/pubmed/31264568
88. Bower W, Schiffer J, Atmar R, Keitel W, Friedlander A, Liu L et al. Use of Anthrax Vaccine in the United States: Recommendations of the Advisory Committee on Immunization Practices, . MMWR. 2019; 68(No. RR-4); 1-14. DOI: http://dx.doi.org/10.15585/mmwr.rr6804a1

5. Briefly describe the biological defence work carried out at the facility, including type(s) of micro-organisms* and/or toxins studied, as well as outdoor studies of biological aerosols.

***Objectives*:** Activities include developing diagnostic assays for public health, developing and validating methods to differentiate and characterize organisms and the toxins that they produce, testing environmental samples for the presence of microorganisms and toxins, and developing environmental sampling methods, conducting molecular and antigenic characterization of organisms, determining pathogenicity and virulence of infectious agents, evaluation of antimicrobial susceptibility, research on

* Including viruses and prions.

potential therapeutics, determining the natnral history of infectious organisms, and conducting epidemiologic studies and surveillance for diseases.

Microorganisms and/or toxins studied: Select Agents (HHS, USDA, Overlap), Select Toxins (HHS), NIAID Category A pathogens

***Outdoor Studies*:** No outdoor studies of biological aerosols were conducted at the facility or off-site by facility personnel.

Form A, Part 2 (iii)

1. What is the name of the facility?
Centers for Disease Control and Prevention (CDC), Deputy Director for Infectious Diseases (DDID), National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Vector Borne Diseases (DVBD) - Ft. Collins

2. Where is it located (include both address and geographical location)?
3156 Rampart Road, Fort Collins, Colorado 80521

3. Floor area of laboratory areas by containment level (m^2):

BSL-2	0 m^2
BSL-3	421 m^2
BSL-4	0 m^2
Total laboratory floor area	421 m^2

4. The organizational structure of each facility.

(i) Total number of personnel 31

(ii) Division of personnel:

Military	0
Civilian	31

(iii) Division of personnel by category:

Scientists	5
Engineers	0
Technicians	8
Administrative and support staff	18

(iv) List the scientific disciplines represented in the scientific/engineering staff.
Animal Science, Bacteriology, Bioinformatics, Biological Science, Cell Biology, Ecology, Entomology, Environmental Science, Epidemiology, Genomics, Immunology, Medicine, Microbiology, Molecular Biology, Molecular Diagnostics, Pathology, Public Health, Structural Biology, Veterinary Medicine, Virology

(v) Are contractor staff working in the facility? If so, provide an approximate number.
Yes Nnmber: 2

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
U.S. Department of Health & Human Services

(vii) What are the funding levels for the following programme areas:

Research	$544,402
Development	$0
Test and evaluation	$0
Total	$544,402

(viii) Briefly describe the publication policy of the facility:

Publication is encouraged and managed by editorial and clearance policies conducted at all levels of the Agency. The clearance policy for information products disseminated outside CDC for public use is available online at: http://www.cdc.gov/od/science/policies. CDC also has an internal policy on "Oversight and clearance of dual use research of concern."

(ix) Provide a list of publicly-available papers and reports resulting from the work published during the previous 12 months. (To include authors, titles and full references.)

1. Nelson CA, Murua C, Jones JM, Mohler K, Zhang Y, Wiggins L, et al; Francisella tularensis Transmission by Solid Organ Transplantation, 2017. Emerg Infect Dis. 2019 Apr; 25(4):767-775. doi: 10.3201/eid2504.181807. Epub 2019 Apr 17. PMID:30730826 https://wwwnc.cdc.gov/eid/article/25/4/18-1807_article
2. Harrist A, Cherry C, Kwit N, Bryan K, Pappert R, Petersen J, et al. Francisella tularensis Exposure Among National Park Service Employees During an Epizootic: Devils Tower National Monument, Wyoming, 2015. Vector Borne Zoonotic Dis. 2019 May; 19(5):316-322. doi: 10.1089/vbz.2018.2360. Epub 2018 Dec 1. PMID:30508402 https://www.ncbi.nlm.nih.gov/pubmed/30508402
3. Prentice KW, DePalma L, Ramage JG, Sarwar J, Parameswaran N, Petersen J, et al. Comprehensive Laboratory Evaluation of a Lateral Flow Assay for the Detection of Yersinia pestis. Health Secur. 2019 Nov/Dec; 17(6):439-453. https://www.ncbi.nlm.nih.gov/pubmed/31859568

5. Briefly describe the biological defence work carried out at the facility, including type(s) of micro-organisms* and/or toxins studied, as well as outdoor studies of biological aerosols.

***Objectives*:** CDC's Division of Vector Borne Diseases (DVBD) possesses many of the select agents that are on the Department of Health and Human Services (HHS) and HHS/U.S. Department of Agriculture overlap lists. Within CDC, DVBD has the primary responsibility for research on tularemia, plague and alphaviruses. This research involves development of assays for surveillance and detection of each agent and molecular and antigenic characterization.

Microorganisms and/or toxins studied: Select Agents (HHS, Overlap), NIAID Category A pathogens

***Outdoor Studies*:** No outdoor studies of biological aerosols were conducted at the facility or off-site by facility personnel.

* Including viruses and prions.

Form A, Part 2 (iii)

National biological defence research and development programmes

1. What is the name of the facility?
Integrated Research Facility at Rocky Mountain Laboratories (IRF-RML)

2. Where is it located (include both address and geographical location)?
903 South 4th Street, Hamilton, Montana 59840

3. Floor area of laboratory areas by containment level (m^2):

BSL-2	1361 m^2
BSL-3	407 m^2
BSL-4	1145 m^2
Total laboratory floor area	2913 m^2

4. The organizational structure of each facility.

(i) Total number of personnel 120

(ii) Division of personnel:

Military	0
Civilian	120

(iii) Division of personnel by category:

Scientists	69
Engineers	0
Technicians	44
Administrative and support staff	7

(iv) List the scientific disciplines represented in the scientific/engineering staff.
Aerobiology, Animal Science, Bacteriology, Biochemistry, Biological Science, Cell Biology, Entomology, Genetics, Genomics, Immunology, Mass Spectrometry, Microbiology, Microscopy, Molecular Biology, Pathology, Proteomics, Veterinary Medicine, Virology

(v) Are contractor staff working in the facility? If so, provide an approximate number.
Yes Number: 6

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
Department of Health and Human Services (HHS)

(vii) What are the funding levels for the following programme areas:

Research	$28,625,007
Development	$0
Test and evaluation	$0
Total	$28,625,007

(viii) Briefly describe the publication policy of the facility:
All researchers are encouraged to publish results in peer-reviewed open literature. The NIH Public Access Policy (http://publicaccess.nih.gov/) ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise form

NIH funds to the National Library of Medicine's PubMed Central digital archive upon acceptance for publication. To help advance science and improve human health, the policy requires that these papers are accessible to the public on PubMed Central no later than 12 months after publication.

(ix) Provide a list of publicly-available papers and reports resulting from the work published during the previous 12 months. (To include authors, titles and full references.)

1. Adney DR, Letko M, Ragan IK, Scott D, van Doremalen N, Bowen RA, et al. Bactrian camels shed large quantities of Middle East respiratory syndrome coronavirus (MERS-CoV) after experimental infection. Emerg Microbes Infect. 2019; 8(1): 717-723. doi:10.1080/22221751.2019.1618687. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1080%2F22221751.2019.1618687
2. Adney DR, Wang L, van Doremalen N, Shi W, Zhang Y, Kong WP, et al. Efficacy of an Adjuvanted Middle East Respiratory Syndrome Coronavirus Spike Protein Vaccine in Dromedary Camels and Alpacas. Viruses. 2019; 11(3). doi:10.3390/v11030212. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.3390%2Fv11030212
3. Avanzato VA, Oguntuyo KY, Escalera-Zamudio M, Gutierrez B, Golden M, Kosakovsky Pond SL, et al.. A structural basis for antibody-mediated neutralization of Nipah virus reveals a site of vulnerability at the fusion glycoprotein apex. Proc Natl Acad Sci U S A. 2019; 116(50): 25057-25067. doi:10.1073/pnas.1912503116. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1073%2Fpnas.1912503116
4. Baiardi S, Redaelli V, Ripellino P, Rossi M, Franceschini A, Moggio M, et al. Prion-related peripheral neuropathy in sporadic Creutzfeldt-Jakob disease. J Neurol Neurosurg Psychiatry. 2019; 90(4): 424-427. doi:10.1136/jnnp-2018-319221. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1136%2Fjnnp-2018-319221
5. Baril L, Valles X, Stenseth NC, Rajerison M, Ratsitorahina M., Pizarro-Cerda J, et al. Can we make human plague history? A call to action. BMJ Global Health. 2019; 4(6), e001984. doi:10.1136/bmjgh-2019-001984. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1136%2Fbmjgh-2019-001984
6. Baskakov IV, Caughey B, Requena JR, Sevillano AM, Surewicz WK, Wille H. The prion 2018 round tables (1): the structure of PrP(Sc). Prion. 2019; 13(1): 46-52. doi:10.1080/19336896.2019.1569450. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1080%2F19336896.2019.1569450
7. Bennett AJ, Bushmaker T, Cameron K, Ondzie A, Niama FR, Parra HJ et al. Corrigendum to Diverse RNA viruses of arthropod origin in the blood of fruit bats suggest a link between bat and arthropod viromes [Virology 528 (2019) 64-72]. Virology. 2019; 528. doi:10.1016/j.virol.2019.01.020. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.virol.2019.01.020
8. Best SM, Ponia SS. Regulation of type I interferon: It's HIP to be K2. Sci Signal. 2019; 12(573). doi:10.1126/scisignal.aaw8549. https://www.ncbi.nlm.nih.gov/pubmed/30890660
9. Boardman K, Rosenke K, Safronetz D, Feldmann H, Schwan TG. Host Competency of the Multimammate Rat Mastomys natalensis Demonstrated by Prolonged Spirochetemias with the African Relapsing Fever Spirochete Borrelia crocidurae. Am J Trop Med Hyg. 2019; 101(6): 1272-1275. doi:10.4269/ajtmh.19-0590. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.4269%2Fajtmh.19-0590
10. Bontemps-Gallo S, Fernandez M, Dewitte A, Raphael E, Gherardini FC, Elizabeth P et al. Nutrient depletion may trigger the Yersinia pestis OmpR-EnvZ regulatory system to promote flea-borne plague transmission. Mol Microbiol. 2019; 112(5): 1471-1482. doi:10.1111/mmi.14372. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1111%2Fmmi.14372
11. Boyle WK, Groshong AM, Drecktrah D, Boylan JA, Gherardini FC, Blevins JS, et al. DksA Controls the Response of the Lyme Disease Spirochete Borrelia burgdorferi to Starvation. J Bacteriol. 2019; 201(4). doi:10.1128/jb.00582-18. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2Fjb.00582-18

12. Carroll JA, Chesebro B. Neuroinflammation, Microglia, and Cell-Association during Prion Disease. Viruses, 2019; 11(1). doi:10.3390/v11010065. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.3390%2Fv11010065
13. Caughey B, Kraus A. Transmissibility versus Pathogenicity of Self-Propagating Protein Aggregates. Viruses. 2019; 11(11). doi:10.3390/v11111044. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.3390%2Fv11111044
14. Chiramel AI, Meyerson NR, McNally KL, Broeckel RM, Montoya VR, Mendez-Solis O. et al. TRIM5alpha Restricts Flavivirus Replication by Targeting the Viral Protease for Proteasomal Degradation. Cell Rep. 2019; 27(11): 3269-3283.e3266. doi:10.1016/j.celrep.2019.05.040. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.celrep.2019.05.040
15. Chong A, Starr T, Finn CE, Steele-Mortimer O. A role for the Salmonella Type III Secretion System 1 in bacterial adaptation to the cytosol of epithelial cells. Mol Microbiol 2019; 112(4): 1270-1283. doi:10.1111/mmi.14361. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1111%2Fmmi.14361
16. Chouikha I, Sturdevant DE, Jarrett C, Sun YC, Hinnebusch BJ. Differential Gene Expression Patterns of Yersinia pestis and Yersinia pseudotuberculosis during Infection and Biofilm Formation in the Flea Digestive Tract. mSystems. 2019; 4(1). doi:10.1128/mSystems.00217-18. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2FmSystems.00217-18
17. de Wit E, Feldmann F, Horne E, Okumura A, Cameroni E, Haddock E et al. Prophylactic efficacy of a human monoclonal antibody against MERS-CoV in the common marmoset. Antiviral Res.2019; 163: 70-74. doi:10.1016/j.antiviral.2019.01.016. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.antiviral.2019.01.016
18. do Carmo Ferreira N, Caughey B. Cell-free prion protein conversion assays in screening for anti-prion drug candidates. Curr Opin Pharmacol. 2019; 44: 1-7. doi:10.1016/j.coph.2018.10.001. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.coph.2018.10.001
19. Edenborough KM, Bokelmann M, Lander A, Couacy-Hymann E, Lechner J, Drechsel O et al. Dendritic Cells Generated From Mops condylurus, a Likely Filovirus Reservoir Host, Are Susceptible to and Activated by Zaire Ebolavirus Infection. Front Immunol. 2019; 10: 2414. doi:10.3389/fimmu.2019.02414. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.3389%2Ffimmu.2019.02414
20. Evans AB, Peterson KE. Throw out the Map: Neuropathogenesis of the Globally Expanding California Serogroup of Orthobunyaviruses. Viruses. 2019; 11(9). doi:10.3390/v11090794. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.3390%2Fv11090794
21. Evans AB, Winkler CW, Peterson KE. Differences in Neuropathogenesis of Encephalitic California Serogroup Viruses. Emerg Infect Dis. 2019; 25(4): 728-738. doi:10.3201/eid2504.181016. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.3201%2Feid2504.181016
22. Faris R, Merling M, Andersen SE, Dooley CA, Hackstadt T, Weber MM. Chlamydia trachomatis CT229 Subverts Rab GTPase-Dependent CCV Trafficking Pathways to Promote Chlamydial Infection. Cell Rep. 2019; 26(12): 3380-3390.e3385. doi:10.1016/j.celrep.2019.02.079. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.celrep.2019.02.079
23. Favole A, Mazza M, Vallino Costassa E, D'Angelo A, Lombardi G, Marconi P et al. Early and Pre-Clinical Detection of Prion Seeding Activity in Cerebrospinal Fluid of Goats using Real-Time Quaking-Induced Conversion Assay. Sci Rep. 2019; 9(1): 6173. doi:10.1038/s41598-019-42449-7. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41598-019-42449-7
24. Feldmann F, Kobasa D, Embury-Hyatt C, Grolla A, Taylor T, Kiso M et al. Oseltamivir Is Effective against 1918 Influenza Virus Infection of Macaques but Vulnerable to Escape. mBio. 2019; 10(5). doi:10.1128/mBio.02059-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2FmBio.02059-19
25. Feldmann F, Shupert WL, Haddock E, Twardoski B, Feldmann H. Gamma Irradiation as an Effective Method for Inactivation of Emerging Viral Pathogens. Am J Trop Med Hyg 2019; 100(5): 1275-1277. doi:10.4269/ajtmh.18-0937. https://www.ncbi.nlm.nih.gov/pubmed/30860018

26. Gomes-Solecki M, Arnaboldi PM, Backenson PB, Benach JL, Cooper CL, Dattwyler RJ et al. Protective Immunity and New Vaccines for Lyme Disease. Clin Infect Dis. 2019. doi:10.1093/cid/ciz872. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1093%2Fcid%2Fciz872
27. Grabowski JM, Nilsson OR, Fischer ER, Long D, Offerdahl DK, Park Y et al. Dissecting Flavivirus Biology in Salivary Gland Cultures from Fed and Unfed Ixodes scapularis (Black-Legged Tick). mBio. 2019; 10(1). doi:10.1128/mBio.02628-18. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2FmBio.02628-18
28. Hawman DW, Meade-White K, Haddock E, Habib R, Scott D, Thomas T et al. Crimean-Congo Hemorrhagic Fever Mouse Model Recapitulating Human Convalescence. J Virol. 2019; 93(18). doi:10.1128/jvi.00554-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2Fjvi.00554-19
29. Hillman C, Stewart PE, Strnad M, Stone H, Starr T, Carmody A et al. Visualization of Spirochetes by Labeling Membrane Proteins With Fluorescent Biarsenical Dyes. Front Cell Infect Microbiol. 2019; 9: 287. doi:10.3389/fcimb.2019.00287. https://www.ncbi.nlm.nih.gov/pubmed/31482073
30. Hoenen T, Groseth A, Feldmann H. Therapeutic strategies to target the Ebola virus life cycle. Nat Rev Microbiol. 2019; 17(10): 593-606. doi:10.1038/s41579-019-0233-2. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41579-019-0233-2
31. Jessop F, Buntyn R, Schwarz B, Wehrly T, Scott D, Bosio CM. IFN-gamma reprograms host mitochondrial metabolism through inhibition of complex II to control intracellular bacterial replication. Infect Immun. 2019. doi:10.1128/iai.00744-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2Fiai.00744-19
32. Judson SD, Munster VJ. Nosocomial Transmission of Emerging Viruses via Aerosol-Generating Medical Procedures. Viruses. 2019; 11(10). doi:10.3390/v11100940. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.3390%2Fv11100940
33. Jutras BL, Savage CR, Arnold WK, Lethbridge KG, Carroll DW, Tilly K et al. The Lyme disease spirochete's BpuR DNA/RNA-binding protein is differentially expressed during the mammal-tick infectious cycle, which affects translation of the SodA superoxide dismutase. Mol Microbiol. 2019; 112(3): 973-991. doi:10.1111/mmi.14336. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1111%2Fmmi.14336
34. Kachroo P, Eraso JM, Beres SB, Olsen RJ, Zhu L, Nasser W et al. Integrated analysis of population genomics, transcriptomics and virulence provides novel insights into Streptococcus pyogenes pathogenesis. Nat Genet. 2019; 51(3): 548-559. doi:10.1038/s41588-018-0343-1. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41588-018-0343-1
35. Kobayashi SD, DeLeo FR. Towards a Monoclonal Antibody-Based Therapy for Prevention and Treatment of Staphylococcus aureus Infections. J Infect Dis. 2019; 219(6): 848-850. doi:10.1093/infdis/jiy667. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1093%2Finfdis%2Fjiy667
36. Kraus A, Saijo E, Metrick MA 2nd, Newell K, Sigurdson CJ, Zanusso G et al. Seeding selectivity and ultrasensitive detection of tau aggregate conformers of Alzheimer disease. Acta Neuropathol. 2019; 137(4): 585-598. doi:10.1007/s00401-018-1947-3. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1007%2Fs00401-018-1947-3
37. Kuisma E, Olson SH, Camerou KN, Reed PE, Karesh WB, Ondzie AI et al. Correction to 'Long-term wildlife mortality surveillance in northern Congo: a model for the detection of Ebola virus disease epizootics'. Philos Trans R Soc Lond B Biol Sci. 2019; 374(1786), 20190658. doi:10.1098/rstb.2019.0658. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1098%2Frstb.2019.0658
38. Kuisma E, Olson SH, Cameron KN, Reed PE, Karesh WB, Ondzie AI et al. Long-term wildlife mortality surveillance in northern Congo: a model for the detection of Ebola virus disease epizootics. Philos Trans R Soc Lond B Biol Sci. 2019; 374(1782), 20180339. doi:10.1098/rstb.2018.0339. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1098%2Frstb.2018.0339
39. Lara A, Cong Y, Jahrling PB, Mednikov M, Postnikova E, Yu S et al. Peripheral immune response in

the African green monkey model following Nipah-Malaysia virus exposure by intermediate-size particle aerosol. PLoS Negl Trop Dis. 2019; 13(6), e0007454. doi:10.1371/journal.pntd.0007454. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1371%2Fjournal.pntd.0007454

40. Larson CL, Beare PA, Heinzen RA. Dependency of Coxiella burnetii Type 4B Secretion on the Chaperone IcmS. J Bacteriol. 2019; 201(23). doi:10.1128/jb.00431-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2Fjb.00431-19
41. Larson CL, Sandoz KM, Cockrell DC, Heinzen RA. Noncanonical Inhibition of mTORC1 by Coxiella burnetii Promotes Replication within a Phagolysosome-Like Vacuole. mBio, 2019; 10(1). doi:10.1128/mBio.02816-18. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2FmBio.02816-18
42. Lee JH, Hammoud DA, Cong Y, Huzella LM, Castro MA, Solomon J et al. The Use of Large-Particle Aerosol Exposure to Nipah Virus to Mimic Human Neurological Disease Manifestations in the African Green Monkey. J Infect Dis. 2019 doi:10.1093/infdis/jiz502. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1093%2Finfdis%2Fjiz502
43. Lehrer AT, Wong TS, Lieberman MM, Johns L, Medina L, Feldmann F et al. Recombinant subunit vaccines protect guinea pigs from lethal Ebola virus challenge. Vaccine. 2019; 37(47): 6942-6950. doi:10.1016/j.vaccine.2019.06.035. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.vaccine.2019.06.035
44. Lo MK, Feldmann F, Gary JM, Jordan R, Bannister R, Cronin J et al. Remdesivir (GS-5734) protects African green monkeys from Nipah virus challenge. Sci Transl Med. 2019; 11(494). doi:10.1126/scitranslmed.aau9242. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1126%2Fscitranslmed.aau9242
45. Long CM, Beare PA, Cockrell DC, Larson CL, Heinzen RA. Comparative virulence of diverse Coxiella burnetii strains. Virulence. 2019; 10(1), 133-150. doi:10.1080/21505594.2019.1575715. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1080%2F21505594.2019.1575715
46. Lunn TJ, Restif O, Peel AJ, Munster VJ, de Wit E, Sokolow S et al. Dose-response and transmission: the nexus between reservoir hosts, environment and recipient hosts. Philos Trans R Soc Lond B Biol Sci. 2019; 374(1782), 20190016. doi:10.1098/rstb.2019.0016. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1098%2Frstb.2019.0016
47. Malachowa N, Kobayashi SD, Lovaglio J, DeLeo FR. Monse Model of Staphylococcus anreus Skin Infection. Methods Mol Biol. 2019; 1960: 139-147. doi:10.1007/978-1-4939-9167-9_12. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1007%2F978-1-4939-9167-9_12
48. Malachowa N, Kobayashi SD, Porter AR., Freedman B, Hanley PW., Lovaglio J et al. Vaccine Protection against Multidrug-Resistant Klebsiella pneumoniae in a Nonhuman Primate Model of Severe Lower Respiratory Tract Infection. mBio. 2019; 10(6). doi:10.1128/mBio.02994-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2FmBio.02994-19
49. Marzi A, Menicucci AR, Engelmann F, Callison J, Horne EJ, Feldmann F et al. Protection Against Marburg Virus Using a Recombinant VSV-Vaccine Depends on T and B Cell Activation. Front Immunol. 2019 Jan 22;9:3071. doi:10.3389/fimmu.2018.03071. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.3389%2Ffimmu.2018.03071
50. Marzi A, Reynolds P, Mercado-Hernandez R, Callison J, Feldmann F, Rosenke R et al. Single low-dose VSV-EBOV vaccination protects cynomolgus macaques from lethal Ebola challenge. EBioMedicine. 2019; 49: 223-231. doi:10.1016/j.ebiom.2019.09.055. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.ebiom.2019.09.055
51. Matz KM, Marzi A, Feldmann H. Ebola vaccine trials: progress in vaccine safety and immunogenicity. Expert Rev Vaccines. 2019; 18(12), 1229-1242. doi:10.1080/14760584.2019.1698952. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1080%2F14760584.2019.1698952
52. Menicucci AR, Jankeel A, Feldmann H, Marzi A., Messaoudi I. Antiviral Innate Responses Induced by VSV-EBOV Vaccination Contribute to Rapid Protection. mBio. 2019: 10(3).

doi:10.1128/mBio.00597-19. https://www.ncbi.nlm.nih.gov/pubmed/31138743
53. Metrick MA 2nd, do Carmo Ferreira N, Saijo E., Hughson AG, Kraus A, Orru C et al. Million-fold sensitivity enhancement in proteopathic seed amplification assays for biospecimens by Hofmeister ion comparisons. Proc Natl Acad Sci U S A. 2019; 116(46): 23029-23039. doi:10.1073/pnas.1909322116. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1073%2Fpnas.1909322116
54. Miller HE, Hoyt FH, Heinzen RA. Replication of Coxiella burnetii in a Lysosome-Like Vacuole Does Not Require Lysosomal Hydrolases. Infect Immun. 2019; 87(11). doi:10.1128/iai.00493-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2Fiai.00493-19
55. Mlera L, Bloom ME. Differential Zika Virus Infection of Testicular Cell Lines. Viruses. 2019; 11(1). doi:10.3390/v11010042. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.3390%2Fv11010042
56. Moormeier DE, Sandoz KM, Beare PA, Sturdevant DE, Nair V, Cockrell DC et al. Coxiella burnetii RpoS Regulates Genes Involved in Morphological Differentiation and Intracellular Growth. J Bacteriol. 2019; 201(8). doi:10.1128/jb.00009-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2Fjb.00009-19
57. Nilsson OR, Kari L, Steele-Mortimer O. Foodborne infection of mice with Salmonella Typhimurium. PLoS One. 2019; 14(8), e0215190. doi:10.1371/journal.pone.0215190. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1371%2Fjournal.pone.0215190
58. Olson SH, Bounga G, Ondzie A, Bushmaker T, Seifert SN, Kuisma E et al. Lek-associated movement of a putative Ebolavirus reservoir, the hammer-headed fruit bat (Hypsignathus monstrosus), in northern Republic of Congo. PLoS One. 2019; 14(10), e0223139. doi:10.1371/journal.pone.0223139. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1371%2Fjournal.pone.0223139
59. Ooi YS, Majzoub K, Flynn RA, Mata MA, Diep J, Li JK et al. An RNA-centric dissection of host complexes controlling flavivirus infection. Nat Microbiol. 2019; 4(12): 2369-2382. doi:10.1038/s41564-019-0518-2. https://www.ncbi.ulm.nih.gov/pubmed/?term=10.1038%2Fs41564-019-0518-2
60. Opoku-Temeng C, Kobayashi SD, DeLeo FR. Klebsiella pneumoniae capsule polysaccharide as a target for therapeutics and vaccines. Comput Struct Biotechnol J. 2019; 17: 1360-1366. doi:10.1016/j.csbj.2019.09.011. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.csbj.2019.09.011
61. Poliquin G, Funk D, Jones S, Tran K, Ranadheera C, Hagan M et al. Correction to: Impact of intensive care unit supportive care on the physiology of Ebola virus disease in a universally lethal non-human primate model. Intensive Care Med Exp. 2019; 7(1): 66. doi:10.1186/s40635-019-0283-9. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1186%2Fs40635-019-0283-9
62. Poliquin G, Funk D, Jones S, Tran K, Ranadheera C, Hagan M et al. Impact of intensive care unit supportive care on the physiology of Ebola virus disease in a universally lethal non-human primate model. Intensive Care Med Exp. 2019; 7(1), 54. doi:10.1186/s40635-019-0268-8. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1186%2Fs40635-019-0268-8
63. Race B, Williams K, Chesebro B. Transmission studies of chronic wasting disease to transgenic mice overexpressing human prion protein using the RT-QuIC assay. Vet Res. 2019; 50(1): 6. doi:10.1186/s13567-019-0626-2. https://www.ncbi.ulm.nih.gov/pubmed/?term=10.1186%2Fs13567-019-0626-2
64. Raymond GJ, Zhao HT, Race B, Raymond LD, Williams K, Swayze EE et al. Antisense oligonucleotides extend survival of prion-infected mice. JCI Insight. 2019; 5. doi:10.1172/jci.insight.131175. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1172%2Fjci.insight.131175
65. Roberts LM, Evans TJ, Bosio CM. T Cell Metabolism Is Dependent on Anatomical Location within the Lung. Immunohorizons. 2019; 3(9): 433-439. doi:10.4049/immunohorizons.1900063. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.4049%2Fimmunohorizons.1900063
66. Rungelrath V, Kobayashi SD, DeLeo FR. Neutrophils in innate immunity and systems biology-level

approaches. Wiley Interdiscip Rev Syst Biol Med. 2019; 12(1), e1458. doi:10.1002/wsbm.1458. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1002%2Fwsbm.1458

67. Saijo E, Groveman BR, Kraus A, Metrick M, Orru CD, Hughson AG, Caughey B. Ultrasensitive RT-QuIC Seed Amplification Assays for Disease-Associated Tau, alpha-Synuclein, and Prion Aggregates. Methods Mol Biol. 2019; 1873, 19-37. doi:10.1007/978-1-4939-8820-4_2. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1007%2F978-1-4939-8820-4_2
68. Saijo E, Metrick MA 2nd, Koga S, Parchi P, Litvan I, Spina S et al. 4-Repeat tau seeds and templating subtypes as brain and CSF biomarkers of frontotemporal lobar degeneration. Acta Neuropathol. 2020; Jan;139(1):63-77. doi: 10.1007/s00401-019-02080-2. Epub 2019 Oct 16. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1007%2Fs00401-019-02080-2
69. Schountz T, Campbell C, Wagner K, Rovnak J, Martellaro C, DeBuysscher BL et al. Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters. Viruses. 2019; 11(3). doi:10.3390/v11030291. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.3390%2Fv11030291
70. Seifert SN, Letko MC, Bushmaker T, Laing ED, Saturday G, Meade-White K et al. Rousettus aegyptiacus Bats Do Not Support Productive Nipah Virus Replication. J Infect Dis. 2019; doi:10.1093/infdis/jiz429. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1093%2Finfdis%2Fjiz429
71. Shannon JG, Hinnebusch BJ. Intravital Confocal Microscopy of Dermal Innate Immune Responses to Flea-Transmitted Yersinia pestis. Methods Mol Biol. 2019; 2010: 57-68. doi:10.1007/978-1-4939-9541-7_5. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1007%2F978-1-4939-9541-7_5
72. Staffaroni AM, Kramer AO, Casey M, Kang H, Rojas JC, Orru CD et al. Association of Blood and Cerebrospinal Fluid Tau Level and Other Biomarkers With Survival Time in Sporadic Creutzfeldt-Jakob Disease. JAMA Neurol. 2019. doi:10.1001/jamaneurol.2019.1071. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1001%2Fjamaneurol.2019.1071
73. Striebel JF, Race B, Williams K, Carroll JA, Klingeborn M, Chesebro B. Microglia are not required for prion-induced retinal photoreceptor degeneration. Acta Neuropathol Commun. 2019; 7(1): 48. doi:10.1186/s40478-019-0702-x. https://www.ncbi.ulm.nih.gov/pubmed/?term=10.1186%2Fs40478-019-0702-x
74. Tang-Huau TL, Feldmann H, Rosenke K. Animal models for Lassa virus infection. Curr Opin Virol. 2019; 37: 112-117. doi:10.1016/j.coviro.2019.07.005. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.coviro.2019.07.005
75. van Doremalen N, Lambe T, Sebastian S, Bushmaker T, Fischer R, Feldmann F et al. A single-dose ChAdOx1-vectored vaccine provides complete protection against Nipah Bangladesh and Malaysia in Syrian golden hamsters. PLoS Negl Trop Dis. 2019; 13(6), e0007462. doi:10.1371/journal.pntd.0007462. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1371%2Fjournal.pntd.0007462
76. Vinton CL, Magaziner SJ, Dowd KA, Robertson SJ, Amaro-Carambot E, Karmele EP et al. Simian Immunodeficiency Virus Infection of Rhesus Macaques Results in Delayed Zika Virus Clearance. mBio. 2019; 10(6). doi:10.1128/mBio.02790-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2FmBio.02790-19
77. Wang Z, Manca M, Foutz A, Camacho MV, Raymond GJ, Race B et al. Early preclinical detection of prions in the skin of prion-infected animals. Nat Commun. 2019; 10(1): 247. doi:10.1038/s41467-018-08130-9. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41467-018-08130-9
78. Wang Z, Manca M, Foutz A, Camacho MV, Raymond GJ, Race B et al. Publisher Correction: Early preclinical detection of prions in the skin of prion-infected animals. Nat Commun. 2019; 10(1): 640. doi:10.1038/s41467-019-08648-6. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41467-019-08648-6
79. Ward A, Hollister JR, Choi YP, Race B, Williams K, Shoup DW et al. Altered distribution, aggregation, and protease resistance of cellular prion protein following intracranial inoculation. PLoS

One. 2019; 14(7), e0219457. doi:10.1371/journal.pone.0219457. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1371%2Fjournal.pone.0219457
80. Wasik BR, de Wit E, Munster V, Lloyd-Smith JO, Martinez-Sobrido L, Parrish CR. Onward transmission of viruses: how do viruses emerge to cause epidemics after spillover? Philos Trans R Soc Lond B Biol Sci. 2019; 374(1782), 20190017. doi:10.1098/rstb.2019.0017. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1098%2Frstb.2019.0017
81. Williams K, Hughson AG, Chesebro B, Race B. Inactivation of chronic wasting disease prions using sodium hypochlorite. PLoS One. 2019; 14(10), e0223659. doi:10.1371/journal.pone.0223659. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1371%2Fjournal.pone.0223659
82. Winkler CW, Woods TA, Groveman BR, Carmody AB, Speranza EE, Martens CA et al. Neuronal maturation reduces the type I IFN response to orthobunyavirus infection and leads to increased apoptosis of human neurons. J Neuroinflammation. 2019; 16(1): 229. doi:10.1186/s12974-019-1614-1. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1186%2Fs12974-019-1614-1
83. Yinda CK, Seifert SN, Macmenamin P, van Doremalen N, Kim L, Bushmaker T et al. A Novel Field-Deployable Method for Sequencing and Analyses of Henipavirus Genomes From Complex Samples on the MinION Platform. J Infect Dis. 2019.. doi:10.1093/infdis/jiz576. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1093%2Finfdis%2Fjiz576
84. Youseff BH, Brewer TG, McNally KL, Izuogu AO, Lubick KJ, Presloid JB et al. TRAF6 Plays a Proviral Role in Tick-Borne Flavivirus Infection through Interaction with the NS3 Protease. iScience. 2019; 15: 489-501. doi:10.1016/j.isci.2019.05.010. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.isci.2019.05.010

5. Briefly describe the biological defence work carried out at the facility, including type(s) of micro-organisms* and/or toxins studied, as well as outdoor studies of biological aerosols.

Objectives: The Integrated Research Facility at Rocky Mountain Laboratories hosts research dedicated to understanding the mechanisms of pathogenesis of microbial agents associated with or likely to cause serious or lethal human diseases using molecular methods and animal model systems. Research activities include pathogenesis studies, vaccinology, and the development of therapeutic countermeasures and rapid diagnostic assays in support of the civilian biodefense program. More information is available at https://www.niaid.nih.gov/about/rocky-mountain-laboratories.

Microorganisms and/or toxins studied: Select Agents (HHS, Overlap, USDA), NIAID Category A pathogens

Outdoor studies: No outdoor studies of biological aerosols were conducted.

Form A, Part 2 (iii)

National biological defence research and development programmes

1. What is the name of the facility?
Integrated Research Facility at Fort Detrick (IRF-Frederick)

2. Where is it located (include both address and geographical location)?
8200 Research Plaza, Frederick, Maryland 21702

3. Floor area of laboratory areas by containment level (m^2):
BSL-2 878 m^2

* Including viruses and prions.

BSL-3	0 m^2
BSL-4	1305 m^2
Total laboratory floor area	2183 m^2

4. The organizational structure of each facility.

(i) Total number of personnel 91

(ii) Division of personnel:

Military	0
Civilian	91

(iii) Division of personnel by category:

Scientists	22
Engineers	2
Technicians	62
Administrative and support staff	5

(iv) List the scientific disciplines represented in the scientific/engineering staff.

Aerobiology, Aerosol Science, Analytical Biochemistry, Biochemistry, Biological Science, Cell Biology, Genomics, Immunology, Microbiology, Microscopy, Molecular Biology, Molecular Diagnostics, Pathology, Public Health, Veterinary Medicine, Virology

(v) Are contractor staff working in the facility? If so, provide an approximate number.

Yes Number: 88

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?

Department of Health and Human Services (HHS)

(vii) What are the funding levels for the following programme areas:

Research	$24,508,496
Development	$0
Test and evaluation	$0
Total	$24,508,496

(viii) Briefly describe the publication policy of the facility:

All researchers are encouraged to publish results in peer-reviewed open literature. The NIH Public Access Policy (http://publicaccess.nih.gov/) ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise form NIH funds to the National Library of Medicine's PubMed Central digital archive upon acceptance for publication. To help advance science and improve human health, the policy requires that these papers are accessible to the public on PubMed Central no later than 12 months after publication.

(ix) Provide a list of publicly-available papers and reports resulting from the work published during the previous 12 months. (To include authors, titles and full references.)

1. Beigel JH, Aga E, Elie-Turenne MC, Cho J, Tebas P, Clark CL et al. Anti-influenza immune plasma for the treatment of patients with severe influenza A: a randomised, double-blind, phase 3 trial. Lancet Respir Med. 2019; 7(11): 941-950. doi:10.1016/s2213-2600(19)30199-7. https://www.ncbi.nlm.nih.gov/pubmed/31582360

2. Beigel JH, Manosuthi W, Beeler J, Bao Y, Hoppers M, Ruxrungtham K et al. Effect of Oral Oseltamivir on Virological Outcomes in Low-risk Adults With Influenza: A Randomized Clinical Trial. Clin Infect Dis. 2019;. doi:10.1093/cid/ciz634. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1093%2Fcid%2Fciz634
3. Bolay FK, Grandits G, Lane HC, Kennedy SB, Johnson MP, Fallah MP et al. PREVAIL I Cluster Vaccination Study With rVSVDeltaG-ZEBOV-GP as Part of a Public Health Response in Liberia. J Infect Dis. 2019; 219(10): 1634-1641. doi:10.1093/infdis/jiy698. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1093%2Finfdis%2Fjiy698
4. Chen P, Chen H, Monssa M, Cheng J, Li T, Qin J et al. rhIL-15 and anti-PD-L1 combination therapy expands a CXCR3+PD1-/low CD8 T cell subset in SIV-infected rhesus macaques. J Infect Dis. 2019; doi:10.1093/infdis/jiz485. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1093%2Finfdis%2Fjiz485
5. Cornish JP, Moore IN, Perry DL, Lara A, Minai M, Promeneur D et al. Clinical Characterization of Host Response to Simian Hemorrhagic Fever Virus Infection in Permissive and Refractory Hosts: A Model for Determining Mechanisms of VHF Pathogenesis. Viruses. 2019; 11(1). doi:10.3390/v11010067. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.3390%2Fv11010067
6. Davey RT Jr, Fernandez-Cruz E, Markowitz N, Pett S, Babiker AG, Wentworth D et al. Anti-influenza hyperimmune intravenous immunoglobulin for adults with influenza A or B infection (FLU-IVIG): a double-blind, randomised, placebo-controlled trial. Lancet Respir Med. 2019; 7(11): 951-963. doi:10.1016/s2213-2600(19)30253-x. https://www.ncbi.nlm.nih.gov/pubmed/31582358
7. Di Mascio M, Lifson JD, Srinivasula S, Kim I, DeGrange P, Keele BF et al. Evaluation of an antibody to alpha4beta7 in the control of SIVmac239-nef-stop infection. Science. 2019; 365(6457): 1025-1029. doi:10.1126/science.aav6695. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1126%2Fscience.aav6695
8. Dodd LE, Follmann D, Proschan M, Wang J, Malvy D, van Griensven J et al. A meta-analysis of clinical studies conducted during the West Africa Ebola virus disease outbreak confirms the need for randomized control groups. Sci Transl Med. 2019; 11(520). doi:10.1126/scitranslmed.aaw1049. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1126%2Fscitranslmed.aaw1049
9. Ekenberg C, Tang MH, Zucco AG, Murray DD, MacPherson CR, Hu X et al. Association Between Single-Nucleotide Polymorphisms in HLA Alleles and Human Immunodeficiency Virus Type 1 Viral Load in Demographically Diverse, Antiretroviral Therapy-Naive Participants From the Strategic Timing of AntiRetroviral Treatment Trial. J Infect Dis. 2019; 220(8): 1325-1334. doi:10.1093/infdis/jiz294. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1093%2Finfdis%2Fjiz294
10. Keshwara R, Hagen KR, Abreu-Mota T, Papaneri AB, Liu D, Wirblich C et al. A Recombinant Rabies Virus Expressing the Marburg Virus Glycoprotein Is Dependent upon Antibody-Mediated Cellular Cytotoxicity for Protection against Marburg Virus Disease in a Murine Model. J Virol. 2019; 93(6). doi:10.1128/jvi.01865-18. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2Fjvi.01865-18
11. Keshwara R, Shiels T, Postnikova E, Kurup D, Wirblich C, Johnson RF et al. Erratum: Publisher Correction: Rabies-based vaccine induces potent immune responses against Nipah virus. NPJ Vaccines. 2019; 4: 18. doi:10.1038/s41541-019-0112-x. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41541-019-0112-x
12. Keshwara R, Shiels T, Postnikova E, Kurup D, Wirblich C, Johnson RF et al. Rabies-based vaccine induces potent immune responses against Nipah virus. NPJ Vaccines. 2019; 4: 15. doi:10.1038/s41541-019-0109-5. https://www.ncbi.nlm.nih.gov/pubmed/31016033
13. Kuhn JH, Adachi T, Adhikari NK J, Arribas JR, Bah IE, Bausch DG et al. New filovirus disease classification and nomenclature. Nat Rev Microbiol. 2019; 17(5): 261-263. doi:10.1038/s41579-019-0187-4. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41579-019-0187-4
14. Liu DX, Perry DL, DeWald LE, Cai Y, Hagen KR, Cooper TK et al. Persistence of Lassa Virus

Associated With Severe Systemic Arteritis in Convalescing Guinea Pigs (Cavia porcellus). J Infect Dis. 2019; 219(11): 1818-1822. doi:10.1093/infdis/jiy641. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1093%2Finfdis%2Fjiy641

15. Migueles SA, Chairez C, Lin S, Gavil NV, Rosenthal DM, Pooran M et al. Adoptive lymphocyte transfer to an HIV-infected progressor from an elite controller. JCI Insight. 2019; 4(18). doi:10.1172/jci.insight.130664. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1172%2Fjci.insight.130664
16. Mulangu S, Dodd LE, Davey RT Jr, Tshiani Mbaya O, Proschan M, Mukadi D et al. A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics. N Engl J Med. 2019; 381(24): 2293-2303. doi:10.1056/NEJMoa1910993. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1056%2FNEJMoa1910993
17. Revell AD, Wang D, Perez-Elias MJ, Wood R, Tempelman H, Clotet B et al. Predicting Virological Response to HIV Treatment Over Time: A Tool for Settings With Different Definitions of Virological Response. J Acquir Immune Defic Syndr. 2019; 81(2): 207-215. doi:10.1097/qai.0000000000001989. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1097%2Fqai.0000000000001989
18. Sneller MC, Clarridge KE, Seamon C, Shi V, Zorawski MD, Justement JS et al. An open-label phase I clinical trial of the anti-alpha4beta7 monoclonal antibody vedolizumab in HIV-infected individuals. Sci Transl Med. 2019; 11(509). doi:10.1126/scitranslmed.aax3447. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1126%2Fscitranslmed.aax3447
19. Sneller MC, Reilly C, Badio M, Bishop RJ, Eghrari AO, Moses SJ et al. A Longitudinal Study of Ebola Sequelae in Liberia. N Engl J Med. 2019; 380(10): 924-934. doi:10.1056/NEJMoa1805435. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1056%2FNEJMoa1805435
20. Winchester NE, Maldarelli F, Mejia Y, Dee N, Dewar R, Laidlaw E et al. 8-Day Inpatient Directly Observed Therapy for ART Failure: A Tool For Preventiug Unnecessary ART Changes aud Optimizing Adherence Support. Clin Infect Dis. 2019. doi:10.1093/cid/ciz590. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1093%2Fcid%2Fciz590

5. Briefly describe the biological defence work carried out at the facility, including type(s) of micro-organisms* and/or toxins studied, as well as outdoor studies of biological aerosols.

Objectives: The Integrated Research Facility at Fort Detrick in Frederick, Maryland manages, coordinates, and facilitates the conduct of emerging infectious disease and biodefense research to develop vaccines, countermeasures, and improved medical outcomes for patients. Battelle Memorial Institute facilitates research performed at the IRF-Frederick with direction from the IRF Scientific Steering Committee.

Microorganisms and/or Toxins Studied: Select Agents (HHS, Overlap), NIAID Category A pathogens

Outdoor studies: No outdoor studies of biological aerosols were conducted.

* Including viruses and prions.

Form A, Part 2 (iii)

National biological defence research and development programmes

1. What is the name of the facility?
C.W. Bill Young Center for Biodefense and Emerging Infectious Diseases

2. Where is it located (include both address and geographical location)?
9000 Rockville Pike, Bethesda, Maryland 20892

3. Floor area of laboratory areas by containment level (m^2):

BSL-2	2725 m^2
BSL-3	1356 m^2
BSL-4	0 m^2
Total laboratory floor area	4081 m^2

4. The organizational structure of each facility.

(i) Total number of personnel 129

(ii) Division of personnel:

Military	0
Civilian	129

(iii) Division of personnel by category:

Scientists	69
Engineers	0
Technicians	54
Administrative and support staff	6

(iv) List the scientific disciplines represented in the scientific/engineering staff.
Bacteriology, Biological Science, Chemistry, Immunology, Medicine, Microbiology, Molecular Biology, Parasitology, Pathogenesis, Toxicology, Vaccine Evaluation, Virology

(v) Are contractor staff working in the facility? If so, provide an approximate number.
Yes Number: 29

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
Department of Health and Human Services (HHS)

(vii) What are the funding levels for the following programme areas:

Research	$35,024,216
Development	$0
Test and evaluation	$0
Total	$35,024,216

(viii) Briefly describe the publication policy of the facility:
All researchers are encouraged to publish results in peer-reviewed open literature. The NIH Public Access Policy (http://publicaccess.nih.gov/) ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise form

NIH funds to the National Library of Medicine's PubMed Central digital archive upon acceptance for publication. To help advance science and improve human health, the policy requires that these papers are accessible to the public on PubMed Central no later than 12 months after publication.

(ix) Provide a list of publicly-available papers and reports resulting from the work published during the previous 12 months. (To include authors, titles and full references.)

1. Akkina R, Barber DL, Bility MT, Bissig KD, Burwitz BJ, Eichelberg K et al. Small Animal Models for Human Immunodeficiency Virus (HIV), Hepatitis B, and Tuberculosis: Proceedings of an NIAID Workshop. Curr HIV Res. 2019. doi:10.2174/1570162x18666191223114019. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.2174%2F1570162x18666191223114019
2. Altman MO, Angel M, Kosik I, Trovao NS, Zost SJ, Gibbs JS et al. Human Influenza A Virus Hemagglutinin Glycan Evolution Follows a Temporal Pattern to a Glycan Limit. mBio. 2019; 10(2). doi:10.1128/mBio.00204-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2FmBio.00204-19
3. Amaral EP, Costa DL, Namasivayam S, Riteau N, Kamenyeva O, Mittereder L et al. A major role for ferroptosis in Mycobacterium tuberculosis-induced cell death and tissue necrosis. J Exp Med. 2019; 216(3): 556-570. doi:10.1084/jem.20181776. https://www.ncbi.nlm.nih.gov/pubmed/30787033
4. Angeletti D, Kosik I, Santos JJS, Yewdell WT, Boudreau CM, Mallajosyula VVA et al. Outflanking immunodominance to target subdominant broadly neutralizing epitopes. Proc Natl Acad Sci U S A. 2019; 116(27): 13474-13479. doi:10.1073/pnas.1816300116. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1073%2Fpnas.1816300116
5. Banks DA, Ahlbrand SE, Hughitt VK, Shah S, Mayer-Barber KD, Vogel SN et al. Mycobacterium tuberculosis Inhibits Autocrine Type I IFN Signaling to Increase Intracellular Survival. J Immunol. 2019; 202(8): 2348-2359. doi:10.4049/jimmunol.1801303. https://www.ncbi.nlm.nih.gov/pubmed/30833347
6. Barber DL, Sakai S, Kudchadkar RR, Fling SP, Day TA, Vergara JA et al. Tuberculosis following PD-1 blockade for cancer immunotherapy. Sci Transl Med. 2019; 11(475). doi:10.1126/scitranslmed.aat2702. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1126%2Fscitranslmed.aat2702
7. Beerli C, Yakimovich A, Kilcher S, Reynoso GV, Flaschner G, Muller DJ et al. Vaccinia virus hijacks EGFR signalling to enhance virus spread through rapid and directed infected cell motility. Nat Microbiol. 2019; 4(2): 216-225. doi:10.1038/s41564-018-0288-2. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41564-018-0288-2
8. Beites T, O'Brien K, Tiwari D, Engelhart CA, Walters S, Andrews J et al. Plasticity of the Mycobacterium tuberculosis respiratory chain and its impact on tuberculosis drug development. Nat Commun. 2019; 10(1): 4970. doi:10.1038/s41467-019-12956-2. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41467-019-12956-2
9. Campbell RA, Schwertz H, Hottz ED, Rowley JW, Manne BK, Washington AV et al. Human megakaryocytes possess intrinsic antiviral immunity through regulated induction of IFITM3. Blood. 2019; 133(19): 2013-2026. doi:10.1182/blood-2018-09-873984. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1182%2Fblood-2018-09-873984
10. Chea LS, Wyatt LS, Gangadhara S, Moss B, Amara RR. Novel Modified Vaccinia Virus Ankara Vector Expressing Anti-apoptotic Gene B13R Delays Apoptosis and Enhances Humoral Responses. J Virol. 2019; 93(5). doi:10.1128/jvi.01648-18. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2Fjvi.01648-18
11. Collins N, Han SJ, Enamorado M, Link VM, Huang B, Moseman EA et al. The Bone Marrow Protects and Optimizes Immunological Memory during Dietary Restriction. Cell. 2019;178(5): 1088-1101.e1015. doi:10.1016/j.cell.2019.07.049. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.cell.2019.07.049

12. Cowan JE, Malin J, Zhao Y, Seedhom MO, Harly C, Ohigashi I et al. Myc controls a distinct transcriptional program in fetal thymic epithelial cells that determines thymus growth. Nat Commun. 2019; 10(1): 5498. doi:10.1038/s41467-019-13465-y. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41467-019-13465-y
13. Cui X, Xu W, Neupane P, Weiser-Schlesinger A, Weng R, Pockros B et al. Bacillus anthracis lethal toxin, but not edema toxin, increases pulmonary artery pressure and permeability in isolated perfused rat lungs. Am J Physiol Heart Circ Physiol. 2019; 316(5): H1076-h1090. doi:10.1152/ajpheart.00685.2018. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1152%2Fajpheart.00685.2018
14. Daniel-Wayman S, Abate G, Barber DL, Bermudez LE, Coler RN, Cynamon MH et al. Advancing Translational Science for Pulmonary Nontuberculous Mycobacterial Infections. A Road Map for Research. Am J Respir Crit Care Med. 2019; 199(8): 947-951. doi:10.1164/rccm.201807-1273PP. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1164%2Frccm.201807-1273PP
15. Davis MJ, Moyer S, Hoke ES, Sionov E, Mayer-Barber KD, Barber DL et al. Pulmonary Iron Limitation Induced by Exogenous Type I IFN Protects Mice from Cryptococcus gattii Independently of T Cells. mBio. 2019; 10(3). doi:10.1128/mBio.00799-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2FmBio.00799-19
16. Deplanche M, Mouhali N, Nguyen MT, Cauty C, Ezan F, Diot,A et al. Staphylococcus aureus induces DNA damage in host cell. Sci Rep. 2019; 9(1): 7694. doi:10.1038/s41598-019-44213-3. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41598-019-44213-3
17. De Rycker M, Horn D, Aldridge B, Amewu RK, Barry CE 3rd, Buckner FS et al. Setting Our Sights on Infectious Diseases. *ACS Infect Dis.* 2020 Jan 10;6(1):3-13. doi: 10.1021/acsinfecdis.9b00371. Epub 2019 Dec 6. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1021%2Facsinfecdis.9b00371
18. Dersh D, Yewdell JW, Wei J. A SIINFEKL-Based System to Measure MHC Class I Antigen Presentation Efficiency and Kinetics. Methods Mol Biol. 2019; 1988: 109-122. doi:10.1007/978-1-4939-9450-2_9. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1007%2F978-1-4939-9450-2_9
19. Dheda K, Gumbo T, Maartens G, Dooley KE, Murray M, Furin J et al. The Lancet Respiratory Medicine Commission: 2019 update: epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant and incurable tuberculosis. Lancet Respir Med. 2019; 7(9): 820-826. doi:10.1016/s2213-2600(19)30263-2. https://www.ncbi.nlm.nih.gov/pubmed/31486393
20. Diamond MS, Ledgerwood JE, Pierson TC. Zika Virus Vaccine Development: Progress in the Face of New Challenges. Annu Rev Med. 2019; 70: 121-135. doi:10.1146/annurev-med-040717-051127. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1146%2Fannurev-med-040717-051127
21. Dollery SJ, Santiago-Crespo RJ, Chatterjee D, Berger EA. Glycoprotein K8.1A of Kaposi's Sarcoma-Associated Herpesvirus Is a Critical B Cell Tropism Determinant Independent of Its Heparan Sulfate Binding Activity. J Virol. 2019; 93(6). doi:10.1128/jvi.01876-18. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2Fjvi.01876-18
22. Drummond RA, Swamydas M, Oikonomou V, Zhai B, Dambuza IM, Schaefer BC et al. CARD9(+) microglia promote antifungal immunity via IL-1beta- and CXCL1-mediated neutrophil recruitment. Nat Immunol. 2019; 20(5): 559-570. doi:10.1038/s41590-019-0377-2. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41590-019-0377-2
23. D'Souza MP, Adams E, Altman JD, Birnbaum ME, Boggiano C, Casorati G et al. Casting a wider net: Immunosurveillance by nonclassical MHC molecules. PLoS Pathog. 2019' 15(2), e1007567. doi:10.1371/journal.ppat.1007567. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1371%2Fjournal.ppat.1007567
24. Elvina Xavier MA, Liu S, Bugge TH, Torres JB, Mosley M, Hopkins SL et al. Tumor Imaging Using Radiolabeled Matrix Metalloproteinase-Activated Anthrax Proteins. J Nucl Med. 2019; 60(10): 1474-1482. doi:10.2967/jnumed.119.226423. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.2967%2Fjnumed.119.226423
25. Esteves PJ, Abrantes J, Baldauf HM, BenMohamed L, Chen Y, Christensen N et al. Author

Correction: The wide ntility of rabbits as models of human diseases. Exp Mol Med. 2019; 51(7): 71. doi:10.1038/s12276-019-0252-0. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs12276-019-0252-0

26. Fauci A, Erbelding E, Whitehead S, Cassetti MC, Handley FG, Gupta R. Dengue vaccine clinical trials in India - An opportunity to inform the global response to a re-emerging disease challenge. Int J Infect Dis. 2019; 84s,:S4-s6. doi:10.1016/j.ijid.2019.03.016. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.ijid.2019.03.016
27. Fischer ES, Campbell WAt, Liu S, Ghirlando R, Fattah RJ, Bugge TH, Leppla SH. Bismaleimide cross-linked anthrax toxin forms fnnctional octamers with high specificity in tumor targeting. Protein Sci. 2019; 28(6): 1059-1070. doi:10.1002/pro.3613. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1002%2Fpro.3613
28. Gao J, Couzens L, Burke DF, Wan H, Wilson P, Memoli MJ et al. Antigenic Drift of the Influenza A(H1N1)pdm09 Virus Neuraminidase Results in Reduced Effectiveness of A/California/7/2009 (H1N1pdm09)-Specific Antibodies. mBio. 2019; 10(2). doi:10.1128/mBio.00307-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2FmBio.00307-19
29. Goo L, Debbink K, Kose N, Sapparapu G, Doyle MP, Wessel AW et al. A protective human monoclonal antibody targeting the West Nile virus E protein preferentially recognizes matnre virions. Nat Microbiol. 2019; 4(1): 71-77. doi:10.1038/s41564-018-0283-7. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41564-018-0283-7
30. Han A, Czajkowski LM, Donaldson A, Baus HA, Reed SM, Athota RS et al. A Dose-finding Study of a Wild-type Influenza A(H3N2) Virus in a Healthy Volnnteer Human Challenge Model. Clin Infect Dis. 2019; 69(12): 2082-2090. doi:10.1093/cid/ciz141. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1093%2Fcid%2Fciz141
31. Hardy BL, Dickey SW, Plaut RD, Riggins DP, Stibitz S, Otto M, Merrell DS. Corynebacterium pseudodiphtheriticum Exploits Staphylococcus aureus Virulence Components in a Novel Polymicrobial Defense Strategy. mBio. 2019; 10(1). doi:10.1128/mBio.02491-18. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2FmBio.02491-18
32. He L, Le KY, Khan BA, Nguyen TH, Hunt RL, Bae JS et al. Resistance to leukocytes ties benefits of quorum sensing dysfunctionality to biofilm infection. Nat Microbiol. 2019; 4(7): 1114-1119. doi:10.1038/s41564-019-0413-x. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41564-019-0413-x
33. Hickman HD. Slowing blood flow to fight viral infection. Science. 2019; 363(6427): 585-586. doi:10.1126/science.aaw3618. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1126%2Fscience.aaw3618
34. Hoft SG, Sallin MA, Kauffman KD, Sakai S, Ganusov VV, Barber DL. Erratum for Hoft et al., The Rate of CD4 T Cell Entry into the Lungs during Mycobacterium tuberculosis Infection Is Determined by Partial and Opposing Effects of Multiple Chemokine Receptors. Infect Immun. 2019; 87(9). doi:10.1128/iai.00491-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2Fiai.00491-19
35. Hoft SG, Sallin MA, Kauffman KD, Sakai S, Ganusov VV, Barber DL. The Rate of CD4 T Cell Entry into the Lungs during Mycobacterium tuberculosis Infection Is Determined by Partial and Opposing Effects of Multiple Chemokine Receptors. Infect Immun. 2019; 87(6). doi:10.1128/iai.00841-18. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2Fiai.00841-18
36. Hussain S, Turnbull ML, Wise HM, Jagger BW, Beard PM, Kovacikova K et al. Mutation of Influenza A Virus PA-X Decreases Pathogenicity in Chicken Embryos and Can Increase the Yield of Reassortant Candidate Vaccine Viruses. J Virol. 2019; 93(2). doi:10.1128/jvi.01551-18. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2Fjvi.01551-18
37. Jagger BW, Dowd KA, Chen RE, Desai P, Foreman B, Burgomaster KE et al. Protective Efficacy of Nucleic Acid Vaccines Against Transmission of Zika Virus During Pregnancy in Mice. J Infect Dis. 2019; 220(10): 1577-1588. doi:10.1093/infdis/jiz338. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1093%2Finfdis%2Fjiz338

38. Jegaskanda S, Andrews SF, Wheatley AK, Yewdell J W, McDermott AB, Subbarao K. Hemagglutinin head-specific responses dominate over stem-specific responses following prime boost with mismatched vaccines. JCI Insight. 2019; 4(22). doi:10.1172/jci.insight.129035. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1172%2Fjci.insight.129035
39. Johnson TP, Larman HB, Lee MH, Whitehead SS, Kowalak J, Toro C et al. Chronic Dengue Virus Panencephalitis in a Patient with Progressive Dementia with Extrapyramidal Features. Ann Neurol. 2019; 86(5): 695-703. doi:10.1002/ana.25588. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1002%2Fana.25588
40. Jones AT, Shen X, Walter KL, LaBranche CC, Wyatt LS, Tomaras GD et al. HIV-1 vaccination by needle-free oral injection induces strong mucosal immunity and protects against SHIV challenge. Nat Commun. 2019; 10(1): 798. doi:10.1038/s41467-019-08739-4. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41467-019-08739-4
41. Katsafanas GC, Moss B. Specific Anchoring and Local Translation of Poxviral ATI mRNA at Cytoplasmic Inclusion Bodies. J Virol. 2019. doi:10.1128/jvi.01671-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2Fjvi.01671-19
42. Khader SA, Divangahi M, Hanekom W, Hill PC, Maeurer M, Makar KW et al. Targeting innate immunity for tuberculosis vaccination. J Clin Invest. 2019; 129(9): 3482-3491. doi:10.1172/jci128877. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1172%2Fjci128877
43. Kosik I, Angeletti D, Gibbs JS, Angel M, Takeda K, Kosikova M et al. Neuraminidase inhibition contributes to influenza A virus neutralization by anti-hemagglutinin stem antibodies. J Exp Med. 2019; 216(2): 304-316. doi:10.1084/jem.20181624. https://www.ncbi.nlm.nih.gov/pubmed/30683737
44. Kosik I, Yewdell JW. Influenza Hemagglutinin and Neuraminidase: Yin(-)Yang Proteins Coevolving to Thwart Immunity. Viruses. 2019; 11(4). doi:10.3390/v11040346. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.3390%2Fv11040346
45. Krummel M, Blish C, Kuhns M, Cadwell K, Oberst A, Goldrath A et al. Universal Principled Review: A Community-Driven Method to Improve Peer Review. Cell. 2019; 179(7): 1441-1445. doi:10.1016/j.cell.2019.11.029. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.cell.2019.11.029
46. Kuskovsky R, Lloyd D, Arora K, Plotkin BJ, Green JM, Boshoff HI et al. C4-Phenylthio beta-lactams: Effect of the chirality of the beta-lactam ring on antimicrobial activity. Bioorg Med Chem. 2019; 27(20): 115050. doi:10.1016/j.bmc.2019.115050. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.bmc.2019.115050
47. Le KY, Villaruz AE, Zheng Y, He L, Fisher EL, Nguyen TH et al. Role of Phenol-Soluble Modulins in Staphylococcus epidermidis Biofilm Formation and Infection of Indwelling Medical Devices. J Mol Biol. 2019; 431(16): 3015-3027. doi:10.1016/j.jmb.2019.03.030. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.jmb.2019.03.030
48. Li G, Bos S, Tsetsarkin KA, Pletnev AG, Despres P, Gadea G, Zhao RY. The Roles of prM-E Proteins in Historical and Epidemic Zika Virus-mediated Infection and Neurocytotoxicity. Viruses. 2019; 11(2). doi:10.3390/v11020157. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.3390%2Fv11020157
49. Lins RC, Boyer AE, Kuklenyik Z, Woolfitt AR., Goldstein J, Hoffmaster AR et al. Zeptomole per milliliter detection and quantification of edema factor in plasma by LC-MS/MS yields insights into toxemia and the progression of inhalation anthrax. Anal Bioanal Chem. 2019; 411(12): 2493-2509. doi:10.1007/s00216-019-01730-4. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1007%2Fs00216-019-01730-4
50. Liu R, Mendez-Rios JD, Peng C, Xiao W, Weisberg AS, Wyatt LS, Moss B. SPI-1 is a missing host-range factor required for replication of the attenuated modified vaccinia Ankara (MVA) vaccine vector in human cells. PLoS Pathog. 2019;15(5), e1007710. doi:10.1371/journal.ppat.1007710. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1371%2Fjournal.ppat.1007710
51. Liu R, Olano LR, Mirzakhanyan Y, Gershon PD, Moss B. Vaccinia Virus Ankyrin-Repeat/F-Box

Protein Targets Interferon-Induced IFITs for Proteasomal Degradation. Cell Rep. 2019; 29(4): 816-828.e816. doi:10.1016/j.celrep.2019.09.039. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.celrep.2019.09.039

52. Liu Q, Liu Q, Meng H, Lv H, Liu Y, Liu J et al. Staphylococcus epidermidis Contributes to Healthy Maturation of the Nasal Microbiome by Stimulating Antimicrobial Peptide Production. Cell Host Microbe. 2019; 27(1): 68-78.e65. doi:10.1016/j.chom.2019.11.003. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.chom.2019.11.003
53. Malo CS, Hickman HD. Tracing Antiviral CD8(+) T Cell Responses Using In Vivo Imaging. J Immunol. 2019; 203(4): 775-781. doi:10.4049/jimmunol.1900232. https://www.ncbi.nlm.nih.gov/pubmed/31383748
54. Mathur A, Feng S, Hayward JA, Ngo C, Fox D, Atmosukarto II et al. A multicomponent toxin from Bacillus cereus incites inflammation and shapes host outcome via the NLRP3 inflammasome. Nat Microbiol. 2019; 4(2): 362-374. doi:10.1038/s41564-018-0318-0. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41564-018-0318-0
55. McCall RM, Sievers ME, Fattah R, Ghirlando R, Pomerantsev AP, Leppla SH. Bacillus anthracis Virulence Regulator AtxA Binds Specifically to the pagA Promoter Region. J Bacteriol. 2019; 201(23). doi:10.1128/jb.00569-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2Fjb.00569-19
56. Memoli MJ, Han A, Walters KA, Czajkowski L, Reed S, Athota R et al. Influenza A Reinfection in Sequential Human Challenge: Implications for Protective Immunity and "Universal" Vaccine Development. Clin Infect Dis. 2019. doi:10.1093/cid/ciz281. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1093%2Fcid%2Fciz281
57. Momben Abolfath S, Kolberg,M, Karginov VA, Leppla SH, Nestorovich EM. Exploring the Nature of Cationic Blocker Recognition by the Anthrax Toxin Channel. Biophys J. 2019; 117(9): 1751-1763. doi:10.1016/j.bpj.2019.08.041. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.bpj.2019.08.041
58. Morens DM, Taubenberger JK. Making Universal Influenza Vaccines: Lessons From the 1918 Pandemic. J Infect Dis; 2019; 219(Supplement_1): S5-s13. doi:10.1093/infdis/jiy728. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1093%2Finfdis%2Fjiy728
59. Namasivayam S, Kauffman KD, McCulloch JA, Yuan W, Thovarai V, Mittereder LR et al. Correlation between Disease Severity and the Intestinal Microbiome in Mycobacterium tuberculosis-Infected Rhesus Macaques. mBio. 2019; 10(3). doi:10.1128/mBio.01018-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2FmBio.01018-19
60. Nivarthi UK, Tu HA, Delacruz MJ, Swanstrom J, Patel B, Durbin AP et al. Longitudinal analysis of acute and convalescent B cell responses in a human primary dengue serotype 2 infection model. EBioMedicine. 2019; 41: 465-478. doi:10.1016/j.ebiom.2019.02.060. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.ebiom.2019.02.060
61. Oliveira-de-Souza D, Vinhaes CL, Arriaga MB, Kumar NP, Cubillos-Angulo JM, Shi R et al. Molecular degree of perturbation of plasma inflammatory markers associated with tuberculosis reveals distinct disease profiles between Indian and Chinese populations. Sci Rep. 2019; 9(1): 8002. doi:10.1038/s41598-019-44513-8. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41598-019-44513-8
62. Paliga D, Raudzus F, Leppla SH, Heumann R, Neumann S. Lethal Factor Domain-Mediated Delivery of Nurr1 Transcription Factor Enhances Tyrosine Hydroxylase Activity and Protects from Neurotoxin-Induced Degeneration of Dopaminergic Cells. Mol Neurobiol. 2019; 56(5): 3393-3403. doi:10.1007/s12035-018-1311-6. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1007%2Fs12035-018-1311-6
63. Pampusch MS, Haran KP, Hart GT, Rakasz EG, Rendahl AK, Berger EA et al. Rapid Transduction and Expansion of Transduced T Cells with Maintenance of Central Memory Populations. Mol Ther Methods Clin Dev. 2019 Sep 30; 16:1-10. doi: 10.1016/j.omtm.2019.09.007. eCollection 2020 Mar

https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.omtm.2019.09.007
64. Park Y, Ahn YM, Jonnala S, Oh S, Fisher JM, Goodwin MB et al. Inhibition of CorA-Dependent Magnesium Homeostasis Is Cidal in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2019; 63(10). doi:10.1128/aac.01006-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2Faac.01006-19
65. Parsons L, An Y, Qi L, White M, van der Woude R, Hartshorn K et al. Influenza Hemagglutinins H2, H5, H6, and H11 are not Targets of Pulmonary Surfactant Protein D: N-glycan subtypes in host-pathogen interactions. J Virol. 2019. doi:10.1128/jvi.01951-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2Fjvi.01951-19
66. Petermann F, Pekowska A, Johnson CA, Jankovic D, Shih HY, Jiang K et al. The Magnitude of IFN-gamma Responses Is Fine-Tuned by DNA Architecture and the Non-coding Transcript of Ifng-as1. Mol Cell. 2019; 75(6): 1229-1242.e1225. doi:10.1016/j.molcel.2019.06.025. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.molcel.2019.06.025
67. Petitdemange C, Kasturi SP, Kozlowski PA, Nabi R, Quarnstrom CF, Reddy PBJ et al. Vaccine induction of antibodies and tissue-resident CD8+ T cells enhances protection against mucosal SHIV-infection in young macaques. JCI Insight. 2019; 4(4). doi:10.1172/jci.insight.126047. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1172%2Fjci.insight.126047
68. Piewngam P, Otto M. Probiotics to prevent Staphylococcus aureus disease? Gut Microbes; 2020; 11(1):94-101. doi: 10.1080/19490976.2019.1591137. Epub 2019 Mar 26. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1080%2F19490976.2019.1591137
69. Piewngam P, Quinones M, Thirakittiwatthana W, Yungyuen T, Otto M, Kiratisin P. Composition of the intestinal microbiota in extended-spectrum beta-lactamase-producing Enterobacteriaceae carriers and non-carriers in Thailand. Int J Antimicrob Agents. 2019; 53(4): 435-441. doi:10.1016/j.ijantimicag.2018.12.006. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.ijantimicag.2018.12.006
70. Raafat D, Otto M, Reppschlager K, Iqbal J, Holtfreter S. Fighting Staphylococcus aureus Biofilms with Monoclonal Antibodies. Trends Microbiol. 2019; 27(4): 303-322. doi:10.1016/j.tim.2018.12.009. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.tim.2018.12.009
71. Reynoso GV, Shannon JP, Americo JL, Gibbs J, Hickman HD. Growth and Purification of Vaccinia Virus Stocks for MPM Imaging. Methods Mol Biol. 2019; 2023: 287-299. doi:10.1007/978-1-4939-9593-6_18. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1007%2F978-1-4939-9593-6_18
72. Reynoso GV, Weisberg AS, Shannon JP, McManus DT, Shores L, Americo JL, et al. Lymph node conduits transport virions for rapid T cell activation. Nat Immunol. 2019; 20(5): 602-612. doi: 10.1038/s41590-019-0342-0. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41590-019-0342-0
73. Salazar V, Jagger BW, Mongkolsapaya J, Burgomaster KE, Dejnirattisai W, Winkler ES et al. Dengue and Zika Virus Cross-Reactive Human Monoclonal Antibodies Protect against Spondweni Virus Infection and Pathogenesis in Mice. Cell Rep. 2019; 26(6): 1585-1597.e1584. doi:10.1016/j.celrep.2019.01.052. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.celrep.2019.01.052
74. Sardinha-Silva A, Mendonca-Natividade FC, Pinzan CF, Lopes CD, Costa DL, Jacot D et al. The lectin-specific activity of Toxoplasma gondii microneme proteins 1 and 4 binds Toll-like receptor 2 and 4 N-glycans to regulate innate immune priming. PLoS Pathog. 2019; 15(6), e1007871. doi:10.1371/journal.ppat.1007871. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1371%2Fjournal.ppat.1007871
75. Shannon JP, Kamenyeva O, Reynoso GV, Hickman HD. Intravital Imaging of Vaccinia Virus-Infected Mice. Methods Mol Biol. 2019; 2023: 301-311. doi:10.1007/978-1-4939-9593-6_19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1007%2F978-1-4939-9593-6_19
76. Sher A, Kelsall BL. The Colon as a Major Site of Immunoregulation by CD4(+) T Cell Subsets in the

Steady State. J Immunol. 2019; 203(7): 1683-1684. doi:10.4049/jimmunol.1900960. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.4049%2Fjimmunol.1900960

77. Silveira-Mattos PS, Narendran G, Akrami K, Fukutani KF, Anbalagan S, Nayak K et al. Author Correction: Differential expression of CXCR3 and CCR6 on CD4(+) T-lymphocytes with distinct memory phenotypes characterizes tuberculosis-associated immune reconstitution inflammatory syndrome. Sci Rep. 2019; 9(1): 8036. doi:10.1038/s41598-019-44429-3. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41598-019-44429-3
78. Silveira-Mattos PS, Narendran G, Akrami K, Fukutani KF, Anbalagan S, Nayak K et al. Differential expression of CXCR3 and CCR6 on CD4(+) T-lymphocytes with distinct memory phenotypes characterizes tuberculosis-associated immune reconstitution inflammatory syndrome. Sci Rep. 2019; 9(1): 1502. doi:10.1038/s41598-018-37846-3. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41598-018-37846-3
79. Singhania A, Graham CM, Gabrysova L, Moreira-Teixeira L, Stavropoulos E, Pitt JM et al. Transcriptional profiling unveils type I and II interferon networks in blood and tissues across diseases. Nat Commun. 2019; 10(1): 2887. doi:10.1038/s41467-019-10601-6. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41467-019-10601-6
80. Stifter SA, Bhattacharyya N, Sawyer AJ, Cootes TA, Stambas J, Doyle SE et al. Visualizing the Selectivity and Dynamics of Interferon Signaling In Vivo. Cell Rep. 2019; 29(11): 3539-3550.e3534. doi:10.1016/j.celrep.2019.11.021. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.celrep.2019.11.021
81. Strydom N, Gupta SV, Fox WS, Via LE, Bang H, Lee M et al. Tuberculosis drugs' distribution and emergence of resistance in patient's lung lesions: A mechanistic model and tool for regimen and dose optimization. PLoS Med. 2019; 16(4): e1002773. doi:10.1371/journal.pmed.1002773. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1371%2Fjournal.pmed.1002773
82. Swanstrom JA, Nivarthi UK, Patel B, Delacruz MJ, Yount B, Widman DG et al. Beyond Neutralizing Antibody Levels: The Epitope Specificity of Antibodies Induced by National Institutes of Health Monovalent Dengue Virus Vaccines. J Infect Dis. 2019; 220(2): 219-227. doi:10.1093/infdis/jiz109. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1093%2Finfdis%2Fjiz109
83. Tan HX, Jegaskanda S, Juno JA, Esterbauer R, Wong J, Kelly HG et al. Subdominance and poor intrinsic immunogenicity limit humoral immunity targeting influenza HA stem. J Clin Invest. 2019; 129(2): 850-862. doi:10.1172/jci123366. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1172%2Fjci123366
84. Taubenberger JK. Influenza's Newest Trick. mBio. 2019; 10(6). doi:10.1128/mBio.02854-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2FmBio.02854-19
85. Taubenberger JK, Kash JC, Morens DM. The 1918 influenza pandemic: 100 years of questions answered and unanswered. Sci Transl Med. 2019; 11(502). doi:10.1126/scitranslmed.aau5485. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1126%2Fscitranslmed.aau5485
86. Taubenberger JK, Morens DM. The 1918 Influenza Pandemic and Its Legacy. Cold Spring Harb Perspect Med. 2019. doi:10.1101/cshperspect.a038695. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1101%2Fcshperspect.a038695
87. Tsetsarkin KA, Maximova OA, Liu G, Kenney H, Teterina NL, Pletnev AG. Stable and Highly Immunogenic MicroRNA-Targeted Single-Dose Live Attenuated Vaccine Candidate against Tick-Borne Encephalitis Constructed Using Genetic Backbone of Langat Virus. mBio. 2019; 10(2). doi:10.1128/mBio.02904-18. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2FmBio.02904-18
88. Van Rompay KKA, Keesler RI, Ardeshir A, Watanabe J, Usachenko J, Singapuri A et al. DNA vaccination before conception protects Zika virus-exposed pregnant macaques against prolonged viremia and improves fetal outcomes. Sci Transl Med. 2019; 11(523). doi:10.1126/scitranslmed.aay2736. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1126%2Fscitranslmed.aay2736

89. Vinhaes CL, Oliveira-de-Souza D, Silveira-Mattos PS, Nogueira B, Shi R, Wei W et al. Changes in inflammatory protein and lipid mediator profiles persist after antitubercular treatment of pulmonary and extrapulmonary tuberculosis: A prospective cohort study. Cytokine. 2019; 123: 154759. doi:10.1016/j.cyto.2019.154759. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.cyto.2019.154759
90. Vinton CL, Magaziner SJ, Dowd KA, Robertson SJ, Amaro-Carambot E, Karnele EP et al. Simian Immunodeficiency Virus Infection of Rhesus Macaques Results in Delayed Zika Virus Clearance. mBio. 2019; 10(6). doi:10.1128/mBio.02790-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2FmBio.02790-19
91. Vrentas CE, Boggiatto PM, Olsen SC, Leppla SH, Moayeri M. Characterization of the NLRP1 inflammasome response in bovine species. Innate Immun. 2019; 1753425919886649. doi:10.1177/1753425919886649. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1177%2F1753425919886649
92. Walters KA, Zhu R, Welge M, Scherler K, Park JK, Rahil Z et al. Differential Effects of Influenza Virus NA, HA Head, and HA Stalk Antibodies on Peripheral Blood Leukocyte Gene Expression during Human Infection. mBio. 2019; 10(3). doi:10.1128/mBio.00760-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2FmBio.00760-19
93. Wang L, Xu Y, Rogers H, Saidi L, Noguchi CT, Li,H et al. UFMylation of RPL26 links translocation-associated quality control to endoplasmic reticulum protein homeostasis. Cell Res. 2020 Jan; 30(1):5-20. doi: 10.1038/s41422-019-0236-6. Epub 2019 Oct 8. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41422-019-0236-6
94. Wang Z, Soni V, Marrine, G, Kaneko T, Boshoff HIM, Barry CE 3rd, Rhee KY. Mode-of-action profiling reveals glutamine synthetase as a collateral metabolic vulnerability of M. tuberculosis to bedaquiline. Proc Natl Acad Sci U S A. 2019; 116(39): 19646-19651. doi:10.1073/pnas.1907946116. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1073%2Fpnas.1907946116
95. Wasserman S, Louw G, Ramangoaela L, Barber G, Hayes C, Omar SV et al. Linezolid resistance in patients with drug-resistant TB and treatment failure in South Africa. J Antimicrob Chemother. 2019; 74(8): 2377-2384. doi:10.1093/jac/dkz206. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1093%2Fjac%2Fdkz206
96. Wei J, Kishton RJ, Angel M, Conn CS, Dalla-Venezia N, Marcel V et al. Ribosomal Proteins Regulate MHC Class I Peptide Generation for Immunosurveillance. Mol Cell. 2019; 73(6): 1162-1173.e1165. doi:10.1016/j.molcel.2018.12.020. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.molcel.2018.12.020
97. Wei J, Yewdell JW. Flu DRiPs in MHC Class I Immunosurveillance. Virol Sin. 2019; 34(2): 162-167. doi:10.1007/s12250-018-0061-y. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1007%2Fs12250-018-0061-y
98. Wei J, Yewdell JW. Immunoribosomes: Where's there's fire, there's fire. Mol Immunol. 2019; 113: 38-42. doi:10.1016/j.molimm.2017.12.026. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.molimm.2017.12.026
99. Whitehead SS, Pierson TC. Effects of dengue immunity on Zika virus infection. Nature. 2019; 567(7749): 467-468. doi:10.1038/d41586-019-00868-6. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fd41586-019-00868-6
100. Whitehouse AJ, Libardo MDJ, Kasbekar M, Brear PD, Fischer G, Thomas CJ et al. Targeting of Fumarate Hydratase from Mycobacterium tuberculosis Using Allosteric Inhibitors with a Dimeric-Binding Mode. J Med Chem. 2019; 62(23), 10586-10604. doi:10.1021/acs.jmedchem.9b01203. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1021%2Facs.jmedchem.9b01203
101. Xavier ME, Liu S, Leppla SH, Cornelissen B. Pre-labelling versus direct labelling of anthrax proteins for imaging of matrix metalloproteinases activity using DOTA-GA. Nucl Med Biol. 2019; 72-73: 49-54. doi:10.1016/j.nucmedbio.2019.07.007. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.nucmedbio.2019.07.007

102. Xiao Y, Park JK, Williams S, Ramuta M, Cervantes-Medina A, Bristol T et al. Deep sequencing of 2009 influenza A/H1N1 virus isolated from volunteer human challenge study participants and natural infections. Virology. 2019; 534: 96-107. doi:10.1016/j.virol.2019.06.004. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.virol.2019.06.004
103. Yewdell JW, Dersh D, Fahraeus R. Peptide Channeling: The Key to MHC Class I Immunosurveillance? Trends Cell Biol. 2019; 29(12): 929-939. doi:10.1016/j.tcb.2019.09.004. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.tcb.2019.09.004
104. Zanker DJ, Oveissi S, Tscharke DC, Duan M, Wan S, Zhang X et al. Influenza A Virus Infection Induces Viral and Cellular Defective Ribosomal Products Encoded by Alternative Reading Frames. J Immunol. 2019; 202(12): 3370-3380. doi:10.4049/jimmunol.1900070. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.4049%2Fjimmunol.1900070

5. Briefly describe the biological defence work carried out at the facility, including type(s) of micro-organisms* and/or toxins studied, as well as outdoor studies of biological aerosols.

Objectives: At the C.W. Bill Young Center for Biodefense and Emerging Infectious Diseases, the Laboratory of Infectious Diseases (LID) focuses on vaccine development, host immune response to viruses, and viral molecular biology and genetics. The Laboratory of Parasitic Diseases (LPD) conducts basic and applied research on the prevention, control, and treatment of a variety of parasitic and bacterial diseases of global importance. The Laboratory of Viral Diseases (LVD) carries out investigations on the molecular biology of viruses, the interactions of viruses with host cells, the pathogens of viral diseases, and host defense mechanisms. The Laboratory of Clinical Immunology and Microbiology (LCIM) conducts clinical and basic science, and epidemiologic research into human immunologic, inflammatory, and infectious diseases. More information can be found at http://www.nih.gov/news-events/news-releases/nih-dedicates-cw-bill-young-center-biodefense-emerging-infectious-diseases.

Microorganisms and/or toxins studied: Select Agents (HHS, USDA), NIAID Category A pathogen

Outdoor studies: No outdoor studies of biological aerosols were conducted.

* Including viruses and prions.

Form A, Part 2 (iii)

National biological defence research and development programmes

1. What is the name of the facility?
Dale and Betty Bumpers Vaccine Research Center (VRC)

2. Where is it located (include both address and geographical location)?
9000 Rockville Pike, Bethesda, Maryland 20892

3. Floor area of laboratory areas by containment level (m^2):

BSL-2	104m^2
BSL-3	0m^2
BSL-4	0m^2
Total laboratory floor area	104 m^2

4. The organizational structure of each facility.

(i) Total number of personnel 13

(ii) Division of personnel:

Military	0
Civilian	13

(iii) Division of personnel by category:

Scientists	13
Engineers	0
Technicians	0
Administrative and support staff	0

(iv) List the scientific disciplines represented in the scientific/engineering staff.
Biological Science

(v) Are contractor staff working in the facility? If so, provide an approximate number.
Yes Number: 7

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
Department of Health and Human Services (HHS)

(vii) What are the funding levels for the following programme areas:

Research	$1,468,352
Development	$0
Test and evaluation	$0
Total	$1,468,352

(viii) Briefly describe the publication policy of the facility:
All researchers are encouraged to publish results in peer-reviewed open literature. The NIH Public Access Policy (http://publicaccess.nih.gov/) ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise form

NIH funds to the National Library of Medicine's PubMed Central digital archive upon acceptance for publication. To help advance science and improve human health, the policy requires that these papers are accessible to the public on PubMed Central no later than 12 months after publication.

(ix) Provide a list of publicly-available papers and reports resulting from the work published during the previous 12 months. (To include authors, titles and full references.)

1. Gaudinski MR, Coates EE, Novik L, Widge A, Houser KV, Burch E et al. Safety, tolerability, pharmacokinetics, and immunogenicity of the therapeutic monoclonal antibody mAb114 targeting Ebola virus glycoprotein (VRC 608): an open-label phase 1 study. Lancet. 2019; 393(10174): 889-898. doi:10.1016/s0140-6736(19)30036-4. https://www.ncbi.nlm.nih.gov/pubmed/30686586
2. Sutton MS, Ellis-Connell A, Balgeman AJ, Barry G, Weiler AM, Hetzel SJ et al. CD8beta Depletion Does Not Prevent Control of Viral Replication or Protection from Challenge in Macaques Chronically Infected with a Live Attenuated Simian Immunodeficiency Virus. J Virol. 2019; 93(15). doi:10.1128/jvi.00537-19. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1128%2Fjvi.00537-19

5. Briefly describe the biological defence work carried out at the facility, including type(s) of micro-organisms* and/or toxins studied, as well as outdoor studies of biological aerosols.

Objectives: The mission of the Vaccine Research Center (VRC) is to conduct research that facilitates the development of effective vaccines for human disease. The research focus of the Biodefense Research Section comprises three areas: development of vaccines and antivirals against hemorrhagic fever viruses such as Ebola, Marburg, and Lassa; studies of the mechanism of vaccine-induced immune protection and host immunity to natural infection; basic research to understand the mechanism of virus replication (entry) and neutralization.

Microorganisms and/or toxins studied: No U.S. Select Agents, NIAID Category A pathogens, or applicable simulants were used.

Outdoor studies: No outdoor studies of biological aerosols were conducted.

* Including viruse and prions.

Form A, Part 2 (iii)

National biological defence research and development programmes: Facilities

1. What is the name of the facility?
Foreign Disease-Weed Science Research Unit

2. Where is it located (provide both address and geographical location)?
1301 Ditto Avenue, Fort Detrick, Maryland 21702

3. Floor area of laboratory areas by containment level (m^2):

BSL-2:	105 m^2
BSL-3:	950 m^2
BSL-4:	0 m^2
Total laboratory floor area:	1,055 m^2

4. The organizational structure of each facility:

(i) Total number of personnel: 36

(ii) Division of personnel:

Military	0
Civilian	36

(iii) Division of personnel by category:

Scientists	11
Engineers	0
Technicians	15
Administrative and support staff	10

(iv) List the scientific disciplines represented in the scientific/engineering staff:
Agronomy, Biological Science, Genomics, Horticulture, Bacteriology, Microbial Forensics, Molecular Diagnostics, Plant Biochemistry, Plant Molecular Biology, Plant Pathology, Plant Physiology, Proteomics, Virology, Weed Science

(v) Are contractor staff working in the facility? If so, provide an approximate number:
No

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
U.S. Department of Agriculture (USDA)

(vii) What are the funding levels for the following program areas:

Research	$4,000,000
Development	$0
Test and evaluation	$0
Total	$4,000,000

(viii) Briefly describe the publication policy of the facility:

All scientific research data is available for publication in peer-reviewed publications after review for dual use determination. All scientists are required to have a minimum of two peer-reviewed publications per year (not all publications by these scientists are relevant to this report). They are encouraged to present research at scientific conferences and to publish in books and proceedings. The USDA Agricultural Research Service (ARS) maintains a searchable online database of publications by scientists at this location (available at https://www.ars.usda.gov/research/publications/publications-at-this-location/?modeCode=80-44-05-00.)

(ix) Provide a list of publicly-available papers and reports resulting from the work during the previous 12 months. (To include authors, titles, and full references.):

None published in CY2019

5. Briefly describe the biological defence work carried out at the facility, including type(s) of microorganisms* and/or toxins studied, as well as outdoor studies of biological aerosols:

***Objectives*:** The Foreign Disease-Weed Science Research Unit has two distinct missions united by a common relationship to plant pathology and the unit's BL-3 plant pathogen laboratory and greenhouse containment facilities. 1) The mission of the foreign disease program is to develop techniques for the rapid detection and identification of new and emerging crop pathogens, and to provide fundamental information on emerging pathogens for risk assessment and the development of practical phytosanitary regulations for the import and export of agricultural commodities and germplasm. 2) The mission of the weed biological control program is to collect foreign pathogens overseas from weeds in their native habitat, and to evaluate, characterize and release the pathogens in the U.S. for biological control of introduced weeds, leading to improved, sustainable weed control practices in agricultural systems with reduced dependence on chemical herbicides. Additional information about research projects conducted at this location is available at http://www.ars.usda.gov/research/projects_programs.htm?modecode=80-44-05-00.

***Microorganisms and/or Toxins Studied*:** Select Agents (Plant Protection and Quarantine, PPQ).

***Outdoor Studies*:** No research work is done outdoors with infectious organisms.

* Including viruses and prions.

Form A, Part 2 (iii)

National biological defence research and development programmes: Facilities

1. What is the name of the facility?
National Animal Disease Center (NADC)

2. Where is it located (provide both address and geographical location)?
1920 Dayton Avenue, Ames, Iowa 50010

3. Floor area of laboratory areas by containment level (m^2):

BSL-2:	4,410 m^2
BSL-3:	2,489 m^2
BSL-4:	0 m^2
Total laboratory floor area:	6,899 m^2

In addition, NADC has unique animal biocontainment facilities ranging from ABSL-2 to ABSL-3Ag (highest biocontainment level that can accommodate food producing animals and various wildlife species). Biocontainment enhancements include HEPA-filtered supply air; dual HEPA filtered exhaust; air-tight doors; shower-in/out of each animal room; heat-treated waste; steam-treated rendering for carcasses; stainless steel penning and gating systems; epoxy-coated floors; and epoxy-covered surfaces. NADC also has two large biocontainment buildings that are considered ABSL-2-enhanced.

ABSL-2:	3,467.7 m^2
ABSL-3:	160.5 m^2
ABSL-3Ag:	1,581.6 m^2

Total biocontainment facility floor area: 5209.8 m^2

4. The organizational structure of each facility:

(i) Total number of personnel: 4

(ii) Division of personnel:

Military	0
Civilian	4

(iii) Division of personnel by category:

Scientists	2
Engineers	0
Technicians	1
Administrative and support staff	1

(iv) List the scientific disciplines represented in the scientific/engineering staff:
Animal Science, Biochemistry, Bioinformatics, Biology, Biotechnology, Cell Biology, Clinical Immunology, Computational Biology, Genetics, Genomics, Immunology, Infectious Disease,, Microbiology, Molecular Biology, Pathogenesis, Pathology, Physiology, Statistics, Structural Biology, Vaccine Evaluation, Veterinarian, Veterinary Clinical Research, Veterinary Medicine, Virology

(v) Are contractor staff working in the facility? If so, provide an approximate number:
No

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?

U.S. Department of Agriculture (USDA)

(vii) What are the funding levels for the following program areas:

Research	$5,900,000
Development	$0
Test and evaluation	$0
Total	$5,900,000

(viii) Briefly describe the publication policy of the facility:

All scientific research data is available for publication in peer-reviewed publications after review for dual use determination. All scientists are required to have a minimum of two peer-reviewed publications per year (not all publications by these scientists are relevant to this report). They are encouraged to present research at scientific conferences and to publish in books and proceedings. The USDA Agricultural Research Service (ARS) maintains a searchable online database of publications by scientists at this location (available at https://www.ars.usda.gov/research/publications/publications-at-this-location/?modeCode=50-30-20-00.)

(ix) Provide a list of publicly-available papers and reports resulting from the work during the previous 12 months. (To include authors, titles, and full references.):

1. Boggiatto, PM, Olsen, SC. Tulathromycin treatment does not affect bacterial dissemination or clearance of *Brucella melitensis* 16M following experimental infection of goats. PLoS One. 2019; 14(12):e0226242. http://dx.doi.org/10.1371/journal.pone.0226242.
2. Boggiatto, PM, Schaut, RG, Kanipe, C, Kelly, SM, Narasimhan, B, Jones, DE, et al. Sustained antigen release polyanhydride-based vaccine platform for immunization against bovine brucellosis. Heliyon. 2019; 5(8)e02370. http://dx.doi.org/10.1016/j.heliyon.2019.e02370.
3. Lindahl, J.F, Vrentas, CE, Deka, RP, Hazarika, RA, Rahman, H, Bambal, RG, et al. Brucellosis in India: results of a collaborative workshop to define One Health priorities. 2019. Trop Anim Health Prod. 52(1):387-396. http://dx.doi.org/10.1007/s11250-019-02029-3.

5. Briefly describe the biological defence work carried out at the facility, including type(s) of microorganisms* and/or toxins studied, as well as outdoor studies of biological aerosols:

***Objectives*:** Support the control and eradication of national and international exotic, emerging, zoonotic, and endemic infectious diseases of animals through a comprehensive research program emphasizing basic and applied research in diagnostics, prevention, and control strategies, prediction of disease outbreaks, molecular epidemiology, and understanding disease pathogenesis. Specifically, the research programs aim to produce new research knowledge and technology to: prevent, reduce or eliminate losses from impaired livestock performance,increased deaths, or condemnations; develop more sensitive, specific and rapid diagnostic tests; develop vaccines designed for the control and, when feasible, the eradication of disease; improve our understanding of the ecology and epidemiology of pathogens at the domestic animal-wildlife interface; and improve our understanding of the genetic and pathophysiologic basis of disease and pathogen virulence. This research provides government regulatory agencies and the livestock industries with improved intervention strategies against priority diseases. Additional information about research projects conducted at this location is available at http://www.ars.usda.gov/research/projects_programs.htm?modecode=50-30-20-00.

* Including viruses and prions.

***Microorganisms and/or Toxins Studied*:** Overlap Select Agents

***Outdoor Studies*:** No research work is done outdoors with infectious organisms.

Form A, Part 2 (iii)

National biological defence research and development programmes: Facilities

1. What is the name of the facility?
Southeast Poultry Research Laboratory

2. Where is it located (provide both address and geographical location)?
934 College Station Road, Athens, Georgia 30605

3. Floor area of laboratory areas by containment level (m^2):

BSL-2:	1,138 m^2
BSL-3:	624 m^2
BSL-4:	0 m^2
Total laboratory floor area:	1,762 m^2

4. The organizational structure of each facility:

(i) Total number of personnel: 31

(ii) Division of personnel:

Military	0
Civilian	31

(iii) Division of personnel by category:

Scientists	7
Engineers	0
Technicians	3
Administrative and support staff	21

(iv) List the scientific disciplines represented in the scientific/engineering staff:
Animal Science, Bioinformatics, Biological Science, Biotechnology, Cell Biology, Computational Biology, Epidemiology, Genetics, Genomics, Immunology, Microbiology, Molecular Biology, Molecular Diagnostics, Pathology, Public Health, Vaccinology, Veterinary Medicine, Virology

(v) Are contractor staff working in the facility? If so, provide an approximate number:
No

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
U.S. Department of Agriculture (USDA)
Department of Health and Human Services (HHS)
Department of Defense (DoD) – partly
Non-Profit Associations
Private Sector Companies
Department of State

(vii) What are the funding levels for the following program areas:

Research	$4,600,000
Development	$0

Test and evaluation	$0
Total	$4,600,000

(viii) Briefly describe the publication policy of the facility:
All scientific research data is available for publication in peer-reviewed publications after review for dual use determination. All scientists are required to have a minimum of two peer-reviewed publications per year (not all publications by these scientists are relevant to this report). They are encouraged to present research at scientific conferences and to publish in books and proceedings. The USDA Agricultural Research Service (ARS) maintains a searchable online database of publications by scientists at this location (available at https://www.ars.usda.gov/research/publications/publications-at-this-location/?modeCode=60-40-10-30.)

(ix) Provide a list of publicly-available papers and reports resulting from the work during the previous 12 months. (To include authors, titles, and full references.):

1. Andreychuk, DB, Andriyasov, AV, Nikonova, ZB., Kozlov, AA, Suarez, DL. Armoured exogenous internal control for real-time PCR diagnosis of avian influenza. Avian Pathol. 2019; 48(5):492-498 https://doi.org/10.1080/03079457.2019.1628918
2. Ayala, AJ, Hernandez, SM, Olivier, TL, Welch, CN, Dimitrov, KM, Goraichuk, et al. Experimental Infection and Transmission of Newcastle Disease Vaccine Virus in Four Wild Passerines. Avian Dis. 2019; 63(3): 389-399. https://doi.org/10.1637/11980-092918-Reg.1
3. Butt, SL, Dimitrov, KM, Zhang, J, Wajid, A, Bibi, T, Basharat, A, et al. Enhanced phylogenetic resolution of Newcastle disease outbreaks using complete viral genome sequences from formalin-fixed paraffin-embedded tissue samples. Virus Genes. 2019; 55(4):505-512. https://doi.org/10.1007/s11262-019-01669-9
4. Carnaccini, S, Santos, JJ, Obadan, AO, Pantin Jackwood, MJ, Suarez, DL, Rajão, DS, et al. 2019. Age-dependent pathogenesis of clade 2.3.4.4A H5N2 HPAIV in experimentally infected broad breasted white turkeys. Vet Microbiol. 231:183-190. https://doi.org/10.1016/j.vetmic.2019.03.011
5. Criado, MF, Bertran, K, Lee, D, Killmaster, L, Stephens, CB, Spackman, E, et al. Efficacy of novel recombinant fowlpox vaccine against recent Mexican H7N3 highly pathogenic avian influenza virus. Vaccine. 2019; 37(16):2232-2243. https://doi.org/10.1016/j.vaccine.2019.03.009
6. Dimitrov, KM, Abolnik, C, Afonso, CL, Albina, E, Bahl, J, Berg, M, et al. Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. Infect Genet Evol. 2019; 74:103917. https://doi.org/10.1016/j.meegid.2019.103917
7. Dimitrov, KM, Ferreira, HL, Pantin-Jackwood, MJ, Taylor, TL, Goraichuk, IV, Crossley, BM, et al. Pathogenicity and transmission of virulent Newcastle disease virus from the 2018-2019 California outbreak and related viruses in young and adult chickens. Virology. 2019; 531:203-218. https://doi.org/10.1016/j.virol.2019.03.010
8. Ferreira, H, Suarez, DL. Single-nucleotide polymorphism analysis to select conserved regions for an improved real-time reverse transcription-PCR test specific for Newcastle Disease Virus. Avian Dis. 2019; 63(4):625-633. https://doi.org/10.1637/aviandiseases-D-19-00071
9. Ferreira, HL, Taylor, TL, Absalon, AE, Dimitrov, KM, Cortes-Espinosa, DV, Butt, SL, et al. Presence of Newcastle disease viruses of sub-genotypes Vc and VIn in backyard chickens and in apparently healthy wild birds from Mexico in 2017. Virus Genes. 2019; 55(4):479-489. https://doi.org/10.1007/s11262-019-01663-1
10. Ferreira, HL, Taylor, TL, Dimitrov, KM, Sabra, M., Afonso, CL, Suarez, DL. Virulent Newcastle disease viruses from chicken origin are more pathogenic and transmissible to chickens than viruses normally maintained in wild birds. Vet Microbiol. 2019; 235:25-34. https://doi.org/10.1016/j.vetmic.2019.06.004

11. Goraichuk, IV, Msoffe, PL, Chiwanga, GH, Dimitrov, KM, Afonso, CL, Suarez, DL. First complete genome sequences of a subgenotype Vd Newcastle disease virus isolate. Microbiol Resour Announc. 2019; 8(27):e00436-19. https://doi.org/10.1128/MRA.00436-19
12. Lee, D, Killian, ML, Torchetti, MK, Brown, I, Lewis, N, Berhane, Y, et al. Intercontinental spread of Asian-origin H7 avian influenza viruses by captive bird trade in 1990's. Infect Genet Evol. 2019: 73:146-150. https://doi.org/10.1016/j.meegid.2019.04.028
13. Msoffe, PL, Chiwanga, GH, Cardona, CJ, Miller, PJ, Suarez, DL. Isolation and Characterization of Newcastle Disease Virus from Live Bird Markets in Tanzania. Avian Dis. 2019; 63 (4):634-640. https://doi.org/10.1637/aviandiseases-D-19-00089
14. Muzyka, D, Rula, O, Tkachenko, S, Muzyka, N, Kothe, S, Pishchanskyi, O, et al. Highly pathogenic and low pathogenic avian influenza H5 subtype viruses in wild birds in Ukraine. Avian Dis. 2019; 63(sp1):235-245. https://doi.org/10.1637/11879-042718-ResNote.1
15. Ross, TM, DiNapoli, J, Giel-Moloney, M, Bloom, CE, Bertran, K, Balzli, C, et al. A computationally designed H5 antigen shows immunological breadth of coverage and protects against drifting avian strains. Proc Natl Acad Sci. 2019; 37:2369-2376. https://doi.org/10.1016/j.vaccine.2019.03.018
16. Spackman, E, Malladi, S, Ssematimba, A, Stephens, CB. Assessment of replicate numbers for titrating avian influenza virus using dose-response models. J Vet Diagn Invest. 2019; 31(4):616-619. https://doi.org/10.1177/1040638719853851
17. Stephens, CB, Prosser, DJ, Pantin-Jackwood, MJ, Berlin, AM, Spackman, E. The pathogenesis of H7 highly pathogenic avian influenza viruses in Lesser Scaup (Aythya affinis). Avian Dis. 2019; 63(sp1):230-234. https://doi.org/10.1637/11909-060118-ResNote.1
18. Ssematimba, A, Malladi, S, Hagenaars, TJ, Bonney, PJ, Weaver, JT, Patyk, KA, et al. Estimating within-flock transmission rate parameter for H5N2 highly pathogenic avian influenza virus in Minnesota turkey flocks during the 2015 epizootic. Epidemiol Infect. 2019; 147:e179. https://doi.org/10.1017/S0950268819000633
19. Welch, CN, Shittu, I, Abolnik, C, Ponman, S, Dimitrov, KM, Taylor, TL, et al. Genomic comparison of Newcastle disease viruses isolated in Nigeria between 2002 and 2015 reveals circulation of highly diverse genotypes and spillover into wild birds. Arch Virol. 2019; 164(8):2031-2047. https://doi.org/10.1007/s00705-019-04288-9
20. Youk, S, Lee, D, Ferreira, HL, Afonso, CL, Absalon, AE, Swayne, DE, et al. Rapid evolution of Mexican H7N3 highly pathogenic avian influenza viruses in poultry. PLoS One. 2019; 14(9):e0222457. https://doi.org/10.1371/journal.pone.0222457
21. Youk, S, Lee, D, Leyson, CM, Smith, D, Criado, MF, DeJesus, E, et al. Loss of fitness of Mexican H7N3 highly pathogenic avian influenza virus in mallards after circulating in chickens. J Virol. 2019; 93(14):e00543-19. https://doi.org/10.1128/JVI.00543-19

5. Briefly describe the biological defence work carried out at the facility, including type(s) of microorganisms* and/or toxins studied, as well as outdoor studies of biological aerosols:

***Objectives*:** Provide scientific solutions to national and international exotic, emerging and endemic poultry viral diseases through a comprehensive research program emphasizing basic and applied research in diagnostics, prevention, and control strategies; prediction of disease outbreaks; molecular epidemiology; and understanding of disease pathogenesis. Produce new research knowledge and technology to: prevent, reduce or eliminate losses from impaired poultry livestock performance, increased deaths, or condemnations; develop more sensitive, specific and rapid diagnostic tests; develop vaccines designed for the control and, when feasible, the eradication of disease; improve our understanding of the ecology and epidemiology of viruses at the wild bird-domestic poultry interface; and improve our understanding of the genetic and pathobiological basis of virulence. This research provides

* Including viruses and prions.

government regulatory agencies and the poultry industries with improved intervention strategies against poultry viral diseases. The Laboratory has one research unit that conducts biological defense work: Exotic and Emerging Avian Viral Diseases Research Unit. Additional information about research projects conducted at this location is available at http://www.ars.usda.gov/main/site_main.htm?modecode=60-40-10-00.

***Microorganisms and/or Toxins Studied*:** Select Agents (USDA).

***Outdoor Studies*:** No research work is done outdoors with infectious organisms.

Form A, Part 2 (iii)

National biological defence research and development programmes

1. What is the name of the facility?
Food and Drug Administration White Oak Campus

Note: This facility includes the Center for Biologics Evaluation and Research (CBER) and the Center for Drug Evaluation and Research (CDER). Inclusion of the CDER is an addition in the 2019 CBM report.

(b)(5)

2. Where is it located (include both address and geographical location)?
10903 New Hampshire Avenue, Silver Spring, MD 20993

3. Floor area of laboratory areas by containment level (m^2):

BSL-2	418 m^2
BSL-3	184 m^2
BSL-4	0 m^2
Total laboratory floor area	602 m^2

Note: The BSL-2 laboratory area for this facility includes the total area, including area that is used for research and development unrelated to biological defense.

4. The organizational structure of each facility.

(i) Total number of personnel 71

(ii) Division of personnel:

Military	0
Civilian	71

(iii) Division of personnel by category:

Scientists	51
Engineers	0
Technicians	0
Administrative and support staff	20

(iv) List the scientific disciplines represented in the scientific/engineering staff.
Bacteriology, Biological Science, Biomedical Science, Biotechnology, Biochemistry, Cell Biology, Genetics, Immunology, Microbiology, Molecular Biology, Molecular Diagnostics, Nanotechnology, Virology

(v) Are contractor staff working in the facility? If so, provide an approximate number.
Yes Number: 6

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
Department of Health and Human Services (HHS)

(vii) What are the funding levels for the following programme areas:

Research	$918,315

Development	$0
Test and evaluation	$0
Total	$918,315

(viii) Briefly describe the publication policy of the facility:
FDA staff are encouraged to publish their research results in peer-reviewed scientific journals. The FDA review and clearance policy ensures publications are of high quality and vetted by subject matter experts as well as leadership. In addition, compliance with the public access to federally-funded scientific research (including digital data and publications) is assured by following FDA's data management plan. The policy states that publications must be uploaded to PubMed Central one year after the publication date. Each medical product Center may also have an additional review and clearance policy.

- FDA review and clearance policy: https://www.fda.gov/media/80061/download
- CDER review and clearance policy: https://www.fda.gov/media/72538/download
- FDA Data Management Plan: http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/StaffManualGuides/UCM479268.pdf

(ix) Provide a list of publicly-available papers and reports resulting from the work published during the previous 12 months. (To include authors, titles and full references.)

1. Adams SE, Lee N, Lugovtsev VY, Kan A, Donnelly RP, Ilyushina NA. Effect of influenza H1N1 neuraminidase V116A and I117V mutations on NA activity and sensitivity to NA inhibitors. Antiviral Res. 2019; 169:104539. doi: 10.1016/j.antiviral.2019.104539. https://www.ncbi.nlm.nih.gov/pubmed/31228489
2. Ilyushina NA, Dickensheets H, Donnelly RP. A comparison of interferon gene expression induced by influenza A virus infection of human airway epithelial cells from two different donors. Virus Research. 2019; 264:1-7. doi: 10.1016/j.virusres.2019.02.002. https://www.ncbi.nlm.nih.gov/pubmed/30779949/
3. Ilyushina NA, Komatsu TE, Ince WL, Donaldson EF, Lee N, O'Rear JJ et al. Influenza A virus hemagglutinin mutations associated with use of neuraminidase inhibitors correlate with decreased inhibition by anti-influenza antibodies. Virol J. 2019; 16(1):149. doi: 10.1186/s12985-019-1258-x. https://www.ncbi.nlm.nih.gov/pubmed/31783761
4. McWilliams IL, Kielczewski JL, Ireland DDC, Sykes JS, Lewkowicz AP, Konduru K et al. Pseudovirus rVSVΔG-ZEBOV-GP Infects Neurons in Retina and CNS, Causing Apoptosis and Neurodegeneration in Neonatal Mice. Cell Rep. 2019; 26(7):1718-26.e4. doi: 10.1016/j.celrep.2019.01.069. https://www.ncbi.nlm.nih.gov/pubmed/30759384
5. Ouyang W, Guo P, Takeda K, Fu Q, Fang H, Frucht DM. Erk1/2 inactivation promotes a rapid redistribution of COP1 and degradation of COP1 substrates. Proc Natl Acad Sci USA. 2020. doi: 10.1073/pnas.1913698117. https://www.ncbi.nlm.nih.gov/pubmed/32041890

5. Briefly describe the biological defence work carried out at the facility, including type(s) of micro-organisms* and/or toxins studied, as well as outdoor studies of biological aerosols.

Objectives: Specific research areas include methods, tools, and models to identify correlates of protection, better evaluate medical countermeasure safety and potency, improve food safety, or developing references and methods to aid developers of diagnostic tests or medical countermeasures.

* Including viruses and prions.

Microorganisms and/or Toxins Studied: Select Agents (HHS, USDA), NIAID Category A pathogens, and simulants

Outdoor studies: No outdoor studies of biological aerosols were conducted.

Form A, Part 2 (iii)

National biological defence research and development programmes

1. What is the name of the facility?
Food and Drug Administration College Park Campus

Note: This facility is an addition in the 2019 CBM report.

(b)(5)

2. Where is it located (include both address and geographical location)?
5001 Campus Drive, College Park, MD 20740

3. Floor area of laboratory areas by containment level (m^2):

BL2	304 m^2
BL3	0 m^2
BL4	0 m^2
Total laboratory floor area	304 m^2

4. The organizational structure of each facility.

(i) Total number of personnel 12

(ii) Division of personnel:

Military	0
Civilian	12

(iii) Division of personnel by category:

Scientists	12
Engineers	0
Technicians	0
Administrative and support staff	0

(iv) List the scientific disciplines represented in the scientific/engineering staff.
Chemistry, Biochemistry, Biology, Food Technology, Microbiology, Genomics Microbiology

(v) Are contractor staff working in the facility? If so, provide an approximate number.
Yes. Number: 3, plus 1 visiting scientist

(vi) What is (are) the source(s) of funding for the work conducted in the facility, including indication if activity is wholly or partly financed by the Ministry of Defence?
Department of Health and Human Services (HHS)

(vii) What are the funding levels for the following programme areas:

Research	$759,049
Development	$0
Test and evaluation	$0
Total	$759,049

(viii) Briefly describe the publication policy of the facility:
FDA staff are encouraged to publish their research results in peer-reviewed scientific journals. The FDA review and clearance policy ensures publications are of high quality and vetted by subject matter experts

as well as leadership. In addition, compliance with the public access to federally-funded scientific research (including digital data and publications) is assured by following FDA's data management plan. The policy states that publications must be uploaded to PubMed Central one year after the publication date.

- FDA review and clearance policy: https://www.fda.gov/media/80061/download
- FDA Data Management Plan: (http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/StaffManualGuides/UCM479268.pdf)

(x) Provide a list of publicly-available papers and reports resulting from the work published during the previous 12 months. (To include authors, titles and full references.)

1. Mansfield MJ, Wentz TG, Zhang S, Lee EJ, Dong M, Sharma SK et al. Bioinformatic discovery of a toxin family in Chryseobacterium piperi with sequence similarity to botulinum neurotoxins. Sci Rep. 2019; 9(1):1634. doi: 10.1038/s41598-018-37647-8. https://www.ncbi.nlm.nih.gov/pubmed/30733520
2. Pillai SP, Prentice KW, Ramage JG, DePalma L, Sarwar J, Parameswaran N et al. Rapid Presumptive Identification of Bacillus anthracis Isolates Using the Tetracore RedLine Alert™ Test. Health Security. 2019;17(4):334-43. doi: 10.1089/hs.2019.0038. https://www.ncbi.nlm.nih.gov/pubmed/31433282
3. Prentice KW, DePalma L, Ramage JG, Sarwar J, Parameswaran N, Petersen J et al. Comprehensive Laboratory Evaluation of a Lateral Flow Assay for the Detection of Yersinia pestis. Health Security. 2019;17(6):439-53. doi: 10.1089/hs.2019.0094. https://www.ncbi.nlm.nih.gov/pubmed/31859568

5. Briefly describe the biological defence work carried out at the facility, including type(s) of micro-organisms* and/or toxins studied, as well as outdoor studies of biological aerosols.

Objectives: The FDA's Center for Food Safety and Applied Nutrition (CFSAN) is a national leader in protecting and promoting public health. Biodefense work at CFSAN is aimed at developing the tools essential for testing a broad array of food products for biological threats. The microbial genomics and analytical chemistry techniques developed at CFSAN are available to other Federal agencies charged with forensic investigations.

Microorganisms and/or Toxins Studied: HHS Select Agent and Toxin, NIAID Category A

Outdoor studies: No outdoor studies of biological aerosols were conducted.

* Including viruses and prions.

Form B

BWC - Confidence Building Measure

Exchange of information on outbreaks of infectious diseases and similar occurrences caused by toxins

United States of America

April 15, 2020

Form B

Information on outbreaks of infectious diseases and similar occurrences, that seem to deviate from the normal pattern

Human Disease Events

Canine rabies importation from Egypt to the United States:

In February 2019 the U.S. Centers for Disease Control and Prevention (CDC) received a blood sample from a dog who bit a veterinary technician in Kansas, United States. The test was confirmed positive for rabies and the veterinary technician was given post-exposure prophylaxis. Unfortunately, the dog needed to be euthanized. The dog was part of a group of 26 dogs imported to the U.S. from Egypt via Canada. CDC tracked down the other dogs and placed them in a quarantine for 4 to 6 months to ensure they were not also infected with rabies. The dog was reportedly vaccinated in December 2018 and CDC worked with partners to investigate the information on the vaccine records. In May 2019, CDC announced a temporary ban on dog imports from Egypt in regard to this and two other cases since 2015. The United States eliminated dog rabies in 2007 and is working to prevent its reintroduction. https://www.cdc.gov/importation/bringing-an-animal-into-the-united-states/Egypt-dogs-temp-suspension.html

Multidrug resistant agents – United States:

In April 2019, the U.S. Centers for Disease Control and Prevention (CDC) investigated an outbreak of cases caused by an emerging multidrug-resistant strain of *Salmonella* Newport with decreased susceptibility to azithromycin that was first detected in 2016. The resistance pattern is concerning because the recommended antibiotics may not work. During June 2018–March 2019, 255 infections were reported among U.S. residents, including approximately 60% of infections that were acquired in the United States. Approximately 40% of patients reported visiting Mexico in the week before illness onset. A high proportion of non-travelers ate beef before illness onset (93%) and detection of the outbreak strain in U.S. beef indicates that beef was a likely source of infection in these patients. The outbreak strain was also detected in soft cheese brought back from Mexico, and a previous sample of beef imported from Mexico. The investigation indicated that dairy cattle in both countries could be a source of more infections with this strain. CDC had several calls with authorities in Mexico and Pan American Health Organization during the course of this investigation. https://www.cdc.gov/mmwr/volumes/68/wr/pdfs/mm6833-H.pdf

Human Infection with Influenza A (H1N1):

On May 3, 2019, an adult >65 years of age with multiple underlying medical conditions developed an influenza-like illness in Michigan. The patient sought medical care at an emergency department on May 5; and was admitted to the hospital as a result of their illness and a respiratory specimen obtained. This specimen was forwarded to the Michigan Department of Health and Human Services Bureau of Laboratories as part of routine surveillance activities. Real-time RT-PCR testing conducted at the public health laboratory was positive for a seasonal influenza A (H1N1)pdm09 virus. The specimen was then forwarded to the National Influenza Reference Center in Wisconsin per national surveillance specimen submission guidelines. Genetic sequencing results of the specimen were suggestive of an influenza A (H1N1) variant (A(H1N1)v) virus. The specimen was forwarded to CDC for additional testing. On May 30, CDC confirmed an A (H1N1)v virus using RT-PCR and genome sequence analysis of the specimen.

This is the first and only variant influenza virus identified in the United States during 2019. The patient has completely recovered. While no exposure to swine was reported, the virus had hemagglutinin and neuraminidase gene segments derived from the seasonal human influenza A (H1N1)pdm09 virus that were likely introduced into swine by a recent zoonosis and were closely related to influenza A(H1N1) viruses now circulating in the U.S. swine population.

Influenza A viruses that normally circulate in swine are called variant influenza viruses when isolated from humans. There may be important antigenic and genetic differences between seasonal influenza viruses that circulate worldwide in the human population and influenza viruses that normally circulate in swine. Since reporting of novel influenza A viruses became nationally notifiable in 2005, 22 human infections with A (H1N1)v, including this one, have been confirmed by CDC.

Influenza A viruses in swine do not usually infect humans, but rare human infections have been reported, usually after direct or indirect exposure to pigs. Since 2005, a total of 483 variant virus infections have been identified in the United States. There has been some limited, non-sustained human-to-human transmission of variant influenza viruses, but no ongoing community transmission has been identified.

Information regarding this A (H1N1)v case can be found at:
https://www.cdc.gov/flu/weekly/weeklyarchives2018-2019/Week21.htm.

General information about variant and influenza A viruses in swine are available at:
http://www.cdc.gov/flu/swineflu/index.htm and http://gis.cdc.gov/grasp/fluview/Novel_Influenza.html.

Animal Disease Events

Summary of Reports: In 2019, the United States submitted six World Organization for Animal Health (OIE) immediate reports for animal disease events. These included one low pathogenic notifiable avian influenza report, one rabbit hemorrhagic disease virus report, two infectious hypodermal and hematopoietic necrosis virus reports, one red sea bream iridoviral disease report, and one report for tilapia lake virus.

Event summaries can be found, by country and then year of occurrence, on the OIE website:
http://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home/indexcontent/newlang/en

2019 Immediate OIE Reports:

Avian Influenza (Infection with Avian Influenza Viruses)
Avian influenza (AI) is caused by influenza type A viruses, which can infect poultry (such as chickens, turkeys, pheasants, quail, domestic ducks, geese, and guinea fowl) and are carried by free-flying waterfowl such as ducks, geese, and shorebirds. AI viruses are classified by a combination of two groups of proteins: hemagglutinin or "H" proteins, of which there are 16 (H1-H16), and neuraminidase or "N" proteins, of which there are 9 (N1-N9). Many different combinations of "H" and "N" proteins are possible. Each combination is considered a different subtype, and each subtype can be further sub-classified as different strains. AI viruses are identified by their pathogenicity (low or high)—the ability of a particular virus strain to produce disease in domestic chickens. Any influenza A virus (including H5 and H7 avian influenza viruses) in its high pathogenic form is reportable in birds, but only H5 and H7 low pathogenic avian influenza viral infections in poultry are notifiable as per Chapter 10.4 on avian influenza of the OIE Terrestrial Animal Health Code (2019):
http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_avian_influenza_viruses.htm.

Low Pathogenic Avian Influenza (LPAI), H5N2—California
OIE Immediate Report April 4, 2019—Final Report September 9, 2019

As part of routine H5/H7 AI surveillance, H5 LPAI was detected in a commercial duck and goose breeder flock. The U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) and the California Department of Food and Agriculture completed a comprehensive epidemiological investigation of this event. There were no clinical signs of illness or increased mortality on the premises. Hatchlings were euthanized and brood stock on the affected premises were monitored for clearance of AI virus. Twenty-one days after the first negative samples, the adult birds were moved from the affected barn to another barn on the premises; shipment of disinfected product and hatchlings under permit was allowed at this time. Sentinel ducks were placed in the affected barn then tested for AI virus 3 weeks later. AI virus was not detected in the sentinel birds and the premises was released from quarantine.

Infectious hypodermal and hematopoietic necrosis virus (IHHNV)
IHHNV infects penaeid shrimp and clinical presentation can vary depending on species, life stage, and population of shrimp affected. High mortalities, reduction in food consumption, changes in behavior or appearance, suppressed growth, and subclinical infection can all result from infection with IHHNV.

IHHNV—Texas and Florida
OIE Immediate Report June 13, 2019 — Final Report August 30, 2019

As part of routine sampling, IHHNV was detected in shrimp on two commercial premises in Texas and one commercial premises in Florida. USDA APHIS, the Texas Parks and Wildlife Department, and the Florida Department of Agriculture and Consumer Services completed a comprehensive epidemiological investigation of these events. Clinical signs consisting of delayed growth were seen in shrimp on one of the Texas premises; there were no clinical signs present in the other two affected premises. All affected shrimp were depopulated and their tanks were cleaned and disinfected.

IHHNV—California and New Mexico
OIE Immediate Report November 20, 2019 — Open at the end of 2019

A research facility in California and a commercial grow-out facility in New Mexico that sold shrimp for public consumption were determined to be IHHNV-positive. Both facilities received shrimp from a previously identified infected farm. The shrimp at both facilities had delayed growth and the shrimp at the grow-out facility also had increased mortality; the increased mortality may have been associated with a bacterial co-infection. USDA APHIS, the California Department of Fish and Wildlife, and the New Mexico Department of Agriculture conducted a comprehensive epidemiological investigation of these events. Both premises were quarantined, all shrimp on the premises were depopulated, and the facilities were cleaned and disinfected.

Rabbit Hemorrhagic Disease Virus-2 (RHDV-2)—Washington
OIE Immediate Report July 19, 2019—Open at the end of 2019

Rabbit hemorrhagic disease (RHD) is a highly contagious and fatal disease of rabbits. It is caused by RHD virus (RHDV), a Calicivirus. There are three recognized pathogenic groups: RHDV (aka RHDV1), RHDVa (considered a subtype of the classic RHDV), and RHDV2.

RHDV-2 was identified in non-commercial pet and feral European rabbits (*Oryctolagus cuniculus*) with high mortality rates in Washington. USDA APHIS and the Washington State Department of Agriculture are conducting a comprehensive epidemiological investigation and surveillance in response to this event.

Red Sea Bream Iridoviral Disease (RSIVD)—Missouri
OIE Immediate Report May 14, 2019—Final Report May 14, 2019

RSIVD affects red sea bream (*Pagrus major*) and more than 30 other species of farmed marine fish in the orders Perciformes and Pleuronectiformes. RSIVD is caused by the red sea bream iridovirus (RSIV) and by the infectious spleen and kidney necrosis virus. RSIV-infected fish are lethargic, severely anemic, have pale gills, gill petechiation, and splenomegaly. They may gasp due to the anemia. Mortality varies widely from 0 to 100 percent depending on fish species, age, physiological condition, water temperature, management practices, and environmental conditions.

The USDA APHIS National Veterinary Services Laboratories (NVSL) confirmed RSIV in imported ornamental fish (common clownfish or *Amphiprion ocellaris*) that were confined to an aquarium. The clownfish started showing clinical signs of disease during the quarantine period after arrival at the aquarium. Lionfish (*Pterois volitans*) that shared the same water at the aquarium with the imported clownfish were also found to be infected with RSIV. The infected fish were never released and were always under containment until euthanized.

Tilapia Lake Virus (TiLV)**—Colorado, Idaho, Wyoming**
OIE Immediate Report May 6, 2019—Final Report August 7, 2019

TiLV is a novel orthomyxovirus-like virus of tilapiine cichlids. All age groups are susceptible to infection with mortality ranging from 10 to 90 percent. Clinical signs are often associated with a concurrent bacterial infection and are not pathognomonic for TiLV. Infected fish have non-specific behavioral signs including lethargy and inappetence. External signs may include exophthalmia, corneal opacity, darkening of skin, skin ulcerations or hemorrhages (with scale loss), pale gills, and swollen abdomen. Internally, fish may have fluid accumulation in the coelomic cavity.

TiLV was detected in three multiple-age class commercial farms. USDA APHIS and the State Departments of Agriculture conducted a comprehensive epidemiological investigation of this event with surveillance. The epidemiological investigation determined that clinical signs consistent with TiLV were first observed on the index premises in November 2018. All clinically ill fish cohorts were depopulated. Exposed fish in the same sites were either depopulated or sent to terminal market. The affected holdings were cleaned and disinfected.

Form C

BWC - Confidence Building Measure

Encouragement of Publication of Results and Promotion of Use of Knowledge

United States of America

April 15, 2020

Form C

HealthData.gov https://healthdata.gov/	This site is dedicated to making data **discoverable** and making **valuable** government data available to the public in the hopes of better health outcomes for all. The data is collected and supplied from agencies from the U.S. Department of Health and Human Services as well as state partners. A full list of agencies contributing data is available on the site at: https://healthdata.gov/content/about Datasets/bundles which have been updated on November 2019: https://healthdata.gov/dataset/chemical-effects-biological-systems-ccbs
Department of Health and Human Services - The Data Initiative https://www.hhs.gov/cto/initiatives/data-initiative/index.html	Across the U.S. Department of Health and Human Services (HHS), agencies collect a vast array of data to better understand the health of the nation. More than 2000 data sets collected by HHS for use by researchers and entrepreneurs have been publicly released. The Open Data movement continues to be a focus of the Department in order to make data available for the public to do research, develop applications, and create new products to improve health. The report on *Leveraging the Nation's Healthcare Data* (December 2019) outlines a vision for data sharing at HHS and presents a holistic approach to data sharing and change management necessary to enable a more evidence based organization. The report is available online at: https://www.hhs.gov/sites/default/files/master-future-state-508.pdf
Department of Health and Human Services – Stakeholder Engagement on Open Data *Roundtable on Sharing and Utilizing Health Data for AI Applications* on April 16, 2019; report available at: https://www.hhs.gov/sites/default/files/sharing-and-utilizing-health-data-for-ai-applications.pdf *Roundtable on Balancing Privacy with Health Data Access* on July 15, 2019; report available at: http://reports.opendataenterprise.org/RT2-Privacy-Report-Final.pdf	The U.S. Department of Health and Human Services (HHS) partnered with the independent nonprofit Center for Open Data Enterprise (CODE) to co-host a series of three Roundtables to find ways to improve how health data is shared and utilized for the public good, as follows:

Roundtable on Leveraging Data on the Social Determinants of Health, October 3, 2019; report available at: http://reports.opendataenterprise.org/Leveraging-Data-on-SDOH-Summary-Report-FINAL.pdf	
NIH U.S. National Library of Medicine https://clinicaltrials.gov/	ClinicalTrials.gov is a database of privately and publicly funded clinical studies conducted around the world (currently 328,725 research studies in all 50 U.S. states and in 209 countries). ClinicalTrials.gov allows the registration of clinical studies with human subjects that assess biomedical and/or health outcomes and that conform to: • Any applicable human subject or ethics review regulations (or equivalent); • Any applicable regulations of the national or regional health authority (or equivalent).
2018 Annual Report of the Federal Select Agent Program https://www.selectagents.gov/annualreport2018.html	The *2018 Annual Report of the Federal Select Agent Program*, released in January 2020, summarizes 2018 program data for the Federal Select Agent Program (FSAP), which regulates the possession, use and transfer of biological select agents and toxins so that important work with potentially dangerous and deadly pathogens can be conducted as safely and securely as possible. FSAP is a partnership between HHS's Centers for Disease Control and Prevention and USDA's Animal and Plant Health Inspection Service.
2018 Federal Select Agent Program Inspection Report Processing Annual Summary (29 October, 2019) https://www.selectagents.gov/resources/2018-FSAP-Inspection-Report-Processing-Annual-Summary_508.pdf	A report summarizing timeliness data related to Federal Select Agent Program (FSAP) issued inspection reports for the FSAP January 1, 2018 December 31, 2018.
Federal Select Agent Program (FSAP) Infographics (27 March, 2019) https://www.selectagents.gov/infographics-compliance.html	The Federal Select Agent Program (FSAP) regulates laboratories working with select agents and toxins and helps to ensure that lifesaving research conducted with these potentially dangerous materials is done as safely and securely as possible. FSAP can take action in order to protect public, animal, and plant health, using a number of available options to address any potential risks and bring the entity back into compliance with the regulations.

	The FSAP has developed a new series of infographics in order to describe these processes in more detail. • How the FSAP Addresses Serious Biosafety or Security Concerns • Theft or Loss of a Select Agent or Toxin • Release of a Select Agent or Toxin • About the FSAP
NIH Data Book **https://report.nih.gov/nihdatabook/**	The National Institutes of Health (NIH) Data Book provides basic summary statistics on extramural grants and contract awards, grant applications, the organizations that NIH supports, the trainees and fellows supported through NIH programs, and the national biomedical workforce.
DRAFT NIH Policy for Data Management and Sharing (November 2019) https://osp.od.nih.gov/scientific-sharing/nih-data-management-and-sharing-activities-related-to-public-access-and-open-science/	This Policy applies to all research, funded or conducted in whole or in part by National Institutes of Health (NIH),that results in the generation of scientific data. This includes research funded or conducted by extramural grants, contracts, intramural research projects, or other funding agreements regardless of NIH funding level or funding mechanism. The effective date of this Policy and subsequent implementation deadlines are dependent upon feedback on this proposal. To facilitate public comments, NIH has established a web-portal where people can easily and securely provide their feedback. Responses must be submitted no later than January 10, 2020.

Form E

BWC - Confidence Building Measure

Declaration of legislation, regulations and other measures

United States of America

April 15, 2020

Form E

Relating to	Legislation	Regulations	Other measures*	Amended since last year
(a) Development, production stockpiling, acquisition or retention of microbial or other biological agents, or toxins, weapons, equipment and means of delivery specified in Article I	Yes	Yes	Yes	Yes[1]
(b) Exports of micro-organisms† and toxins	Yes	Yes	Yes	Yes[2]
(c) Imports of micro-organisms‡ and toxins	Yes	Yes	Yes	Yes [3]
(d) Biosafety‡ and biosecurity§	Yes	Yes	Yes	Yes[4]

EXPLANATORY NOTES

[1] (a) Development, production stockpiling, acquisition or retention of microbial or other biological agents, or toxins, weapons, equipment and means of delivery specified in Article I

- **2019-2022 National Health Security Strategy:** Provides a vision for strengthening the United States ability to prevent, detect, assess, prepare for, mitigate, respond to, and recover from 21st century health security threats and to strengthen the U.S. public health and health care systems to effectively and swiftly confront the devastating consequences of health security risks. For example, health security threats including the use of biological weapons, emerging infectious diseases that could lead to a pandemic, or catastrophic natural disasters and human-caused incidents. Read more at: https://www.phe.gov/Preparedness/planning/authority/nhss/Pages/default.aspx

- **Global Health Security Strategy (2019):** Outlines the United States Government's approach to strengthen global health security, including accelerating the capabilities of targeted countries to prevent, detect, and respond to infectious disease. It states that the United States Government will continue to collaborate with partners, including countries, multilateral organizations, and nongovernmental stakeholders through the Global Health Security Agenda (GHSA) to strengthen and sustain capacity to prevent, detect, and respond to infectious disease threats. This includes working with partners to make progress toward achieving 2005 International Health Regulations (IHR) core public health capacities while supporting implementation and compliance with the Biological Weapons Convention (BWC), United Nations Security Council Resolution 1540, World Organization for Animal Health Performance of Veterinary Services Pathway, and other international frameworks. Read more at: https://www.whitehouse.gov/wpcontent/uploads/2019/05/GHSS.pdf

(b)(5)

[2] (b) Exports of micro-organisms and toxins:

- **Revisions to Country Group Designations for Venezuela and Conforming Changes for License Requirements:** In this final rule, the Bureau of Industry and Security (BIS) amends the Export

* Including guidelines.
† Micro-organisms pathogenic to man, animals and plants in accordance with the Convention.
‡ In accordance with the latest version of the WHO Laboratory Biosafety Manual or equivalent national or international guidance.
§ In accordance with the latest version of the WHO Laboratory Biosecurity Guidance or equivalent national or international guidance.

Administration Regulations (EAR) to remove Venezuela from Country Group B and adds Venezuela to Country Groups D:1-4, which list countries of national security concern, as well as of nuclear, chemical and biological weapons, and missile technology concerns. The changes in this final rule also better align the Country Group designations for Venezuela with other EAR national security-related provisions that already apply to Venezuela. Consistent with adding Venezuela to Country Group D:3, a license will now be required for the export or reexport of items subject to Export Control Classification Number (ECCN) 1C991.d to Venezuela. ECCN 1C991.d includes "medical products containing specified human and animal pathogens and "toxins." The license requirements for these items to all other countries have not changed. https://www.bis.doc.gov/index.php/documents/regulations-docs/federal-register-notices/federal-register-2019/2399-84-fr-24018/file

|3| (c) Imports of micro-organisms and toxins:

- **2019 Tools for the CDC Import Permit Program (IPP):** The IPP regulates infectious biological materials coming into the U.S. in order to prevent the introduction and spread of disease in humans. This helps to protect the health of laboratory workers and those in the surrounding communities. CDC developed an interactive e-tool (https://www.cdc.gov/cpr/ipp/etool.htm) and a fact sheet (https://www.cdc.gov/cpr/ipp/docs/DoYouNeedanImportPermitFactSheetFINAL.pdf)that can help potential applicants determine if a CDC import permit is needed. Read more at: https://www.cdc.gov/cpr/ipp/about.htm

|4| (d) Biosafety and biosecurity:

i. Amendments to Select Agent and Toxin Regulations:

- Biennial review: As required by the Bioterrorism Response Act, the Federal Select Agent Program (FSAP) reviews the list of select agents and toxins on at least a biennial basis. FSAP last republished the list of select agents and toxins in the *Federal Register* on January 19, 2017 (82 FR 6278 and 82 FR 6197). In 2019, FSAP initiated the review of the list of select agents and toxins.

ii. Select Agent and Toxin Regulations: Policy statements and regulatory interpretations:

- During 2019, FSAP generated the following policies:
  - Regulatory interpretation regarding requirement for inactivation certificates and intra-entity transfers (https://www.selectagents.gov/reg-int_certificates.html)
  - Regulatory interpretation regarding transferring excluded amounts of toxins (https://www.selectagents.gov/reg-int_excluded-transfer.html)

- Policy Statement on Annual Inspections Required by Section 9(a)(6) of the Select Agent Regulations: On March 22, 2019, FSAP published a policy statement to require a Responsible Official (RO) ensure that an annual inspection is conducted for each registered space where select agents and toxins are stored or used in order to determine compliance with the requirements of the select agent regulations. The policy statement outlines inspection requirements focused on biosafety and biocontainment plans, security plans, incident response plans, and training. The policy statement is effective March 22, 2019, and is available at https://www.selectagents.gov/policystatement_annualinspection.html. It replaces the FSAP Policy Statement: Entity Annual Internal Inspections Required by Section 9(a)(6) of the Select Agent Regulations dated August 9, 2018.

iii. Policy statements and regulatory interpretations concerning Select Agent and Toxin Regulations (Public Health Security and Bioterrorism Preparedness and Response Act of 2002

and the Agricultural Bioterrorism Protection Act of 2002 concerning the Federal Select Agent Program):

- During 2019, the Departments of Health and Human Services (HHS) and Agriculture (USDA) generated the following policies and regulatory interpretations:
    - Federal Select Agent Program Policy Statement: Entity Annual Internal Inspections Required by Section 9(a)(6) of the Select Agent Regulations (March 22, 2019); https://www.selectagents.gov/policystatement_annualinspection.html
    - Regulatory Interpretation regarding requirement for inactivation certificates and intra-entity transfers (May 17, 2019); https://www.selectagents.gov/reg-int_certificates.html
    - Regulatory interpretation regarding transfer of excluded toxins (17 June, 2019); https://www.selectagents.gov/reg-int_excluded-transfer.html

iv. Federal Select Agent Program Security and Biosafety Guidance Documents for the Regulated Community:

- Technical and Non-technical Updates to Resource Documents:

Federal Select Agent Program Responsible Official Resource Manual
The Responsible Official is the individual designated by the registered entity with the authority and responsibility to act on behalf of the entity to ensure compliance with the select agent regulations. In February 2019, FSAP revised the Responsible Official Resource Manual to remove the "Three Year Cycle of RO Tasks" section and reference FSAP's policy and guidance on annual internal inspections. The manual is available at https://www.selectagents.gov/rorm-changes.html.

Exclusion Guidance Document
FSAP oversees the use, possession, and transfer of select agents and toxins at registered entities throughout the United States. The select agent regulations provide criteria for the exclusion of select agents and toxins (See 42 CFR §§ 73.3, 73.4; 9 CFR §§ 121.3, 121.4; 7 CFR § 331.3). The Exclusion Guidance Document provides additional information to entities or individuals who request to exclude attenuated strains of select agents or select toxins modified to be less potent or toxic from the requirements of the select agent regulations. In July 2019, FSAP updated the Exclusion Guidance Document to include eFSAP information and to update links. The document is available at https://www.selectagents.gov/egd-changes.html.

Restricted Experiments Guidance
In July 2019, FSAP updated the Restricted Experiments Guidance to include eFSAP information and to update links. The guidance is available athttps://www.selectagents.gov/re-intro.html.

v. Amendments to the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*):

- In April 2019, the Department of Health and Human Services (HHS)/ National Institutes of Health (NIH) published revised NIH Guidelines to eliminate duplicative review and reporting requirements for human gene transfer protocols and to refocus the role of the NIH Recombinant DNA Advisory Committee (RAC)- now renamed the Novel and Exceptional Technology and Research Advisory Committee (NExTRAC)- to be closer to its original mandate a transparent forum for science, safety, and ethics of emerging biotechnologies. The document is available at: https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf

- Additional Guidance on incident reporting (May 2019): The *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*) states that "…any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses" must be reported to NIH within 30 days. Additional guidance on incident reporting, including FAQs and an incident reporting template, can be found here: https://osp.od.nih.gov/biotechnology/faqs-on-incident-reporting/

vi. Other Measures to Advance Biosafety and Biosecurity in the United States:

- US Government Guidance on *Managing Solid Waste Contaminated with a Category A Infectious Substance* (August *2019);* read more at: https://www.phe.gov/s3/BioriskManagement/biocontainment/Pages/waste-management.aspx

- 23 September 2019: CDC Received Designation as PAHO/WHO Collaborating Centre for Biosafety and Biosecurity; read more at: https://www.cdc.gov/media/releases/2019/p0923-collaborating-centre.html

- Under the HHS *Framework for Guiding Funding Decisions about Proposed Research Involving Enhanced Potential Pandemic Pathogens* (P3CO Framework)- https://www.phe.gov/s3/dualuse/Documents/p3co.pdf HHS/ASPR led the multidisciplinary pre-funding review of research projects involving potential pandemic pathogens (PPPs). Read more at: https://www.phe.gov/s3/dualuse/Pages/Two-Research-Projects-PPP.aspx

- FBI Enforcement of BWC Article I: The United States established the Biological Weapons Anti-terrorism (BWAT) Act in 1989, as an instrument of implement Article I of the BWC and violations are investigated and prosecuted as federal crimes. The BWAT Act was codified in the U.S. federal criminal code (Title 18 of the United States Code, Section 175(a), 175(b), and 175b; also referred to as 18 USC 175). As a result, individual(s) in the United States can be charged with a federal crime if they use a biological agent, toxin, or delivery system as a weapon, or are in possession of any biological agent without a justifiable research or peaceful purpose. It is also a crime to knowingly possess a Select Agent or toxin, regardless of intent, if the individual does not have legitimate access (registered with the U.S. Federal Select Agent Program) and purpose. In 2019, the FBI responded to several incidents that involved biological material and led investigations that resulted in the prosecutions for the violation of 18 USC 175.

- Amendment to Title 18 USC 175B: In July 2019, an amendment to 18 USC 175b was signed into law, known as the Effective Prosecution of Possession of Biological Toxins and Agents Act of 2019 (https://www.congress.gov/116/plaws/publ31/PLAW-116publ31.pdf). This corrected a previous oversight excluding a number of toxins affecting the effective prosecution of individuals, including ricin and tetrodotoxin.

- FBI Security Risk Assessments (SRAs) – 3,629 SRAs Completed in 2019: The FBI conducts Security Risk Assessments (SRAs), a requirement of the U.S. Federal Select Agent Program (FSAP), on all entities and personnel in the United States requesting possession, use, or transfer of biological select agents and toxins (BSAT). Using various biographical and biometric databases, the FBI determines if a candidate meets the criteria of a "restricted person" based upon a list of prohibitors found under 18 U.S. Code 175b (derived from the USA PATRIOT Act and the Public Health Security and Bioterrorism Preparedness and Response Act). In 2019, 3,629 SRAs were processed by the FBI (Criminal Justice Information Services Division, Bioterrorism Risk Assessment Group). Of the 3,629

individual SRAs processed, 30 BSAT access candidates were determined to meet the criteria of a "restricted person." The FBI's adjudication is provided to the Department of Health and Human Services or the Department of Agriculture, who decides whether to grant or deny the requesting entity or individual access to BSAT.

- FBI Biosecurity Outreach: During 2019, the FBI conducted over twenty biosecurity outreach events at public and private research institutions across the UnitedStates. These engagements provided an environment where law enforcement (the FBI and State and local law enforcement agencies) and the research communities (research students, professors/researchers, biosafety officers, etc.) could engage in mutually beneficial dialogue. During these events, the FBI aimed to: 1) improve situational awareness of biosecurity threats, and 2) foster a mechanism for to report suspicious activities. The FBI works to enhance the science community's awareness of threats and vulnerabilities, both internal and external, as scientists could be exploited because of their expertise and access to biological material/technologies. The FBI also educates the science community of the FBI's roles and responsibilities in the biosecurity arena and provides resources that can be used to mitigate suspicious activities.

  In addition, the FBI conducted biosecurity engagements with both the international synthetic biology sector and the amateur biology community in the United States. Engagements include attendance at conferences and regional meetings, FBI-sponsored national and international workshops, assistance in the development of safety and security framework, and dissemination of education materials. For example, during 2019, the FBI participated in security discussions with domestic and international synthetic biology stakeholders, as well as sponsored and conducted biosecurity outreach at the 2019 International Genetically Engineered Machine Competition, the largest, annual synthetic biology meeting of undergraduate students worldwide. The FBI also provided training on Food Defense and on multi-sectoral approaches to determine if disease outbreaks in human, plant, and animal could be other than natural occurrences.

Form F

BWC - Confidence Building Measure

Declaration of Past Activities in Offensive and/or Defensive Biological Research and Development Programmes

United States of America

April 15, 2020

Form F

Declaration of Past Activities in Offensive and/or Defensive Biological Research and Development Programmes

1. **Date of entry into force of the Convention for the State party**
   26 March 1975

2. **Past offensive biological research and development programmes:**
   Nothing new to declare

Form G

BWC - Confidence Building Measure

Declaration of Vaccine Production Facilities

United States of America

April 15, 2020

Form G

Declaration of vaccine production facilities

The U.S. Food and Drug Administration publishes a current list of human vaccines licensed in the United States, including associated production facilities. This list is available at: http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm093833.htm.

Data provided on CBM Form G are excerpted from the publicly available website listed above (as accessed on February 14, 2020). Trade names are included when provided by the manufacturer. Specific and current information about a vaccine, and contact information for the manufacturer, are available by following the hyperlinks provided on the above website.

Form G

Declaration of vaccine production facilities

1. **Name of facility**
   Barr Laboratories, Inc.

2. **Location (Mailing Address)**
   1235 Mays Mill Road,
   Forrest, Virginia 24551

3. **General description of the types of diseases covered:**
   Acute respiratory disease caused by Adenovirus Type 4 and Type 7

   Vaccines:
   - Adenovirus Type 4 and Type 7 Vaccine, Live, Oral

Form G

Declaration of vaccine production facilities

1. Name of facility
Emergent Biosolutions

2. Location (Mailing Address)
3500 N. Martin Luther King Jr. Blvd.
Lansing, Michigan 48906

3. General description of the types of diseases covered:
Anthrax disease caused by *Bacillus anthracis* and smallpox disease

Vaccines:
- Anthrax Vaccine Adsorbed - [Biothrax]
- Smallpox (Vaccinia) Vaccine, Live -[ACAM2000]

Form G

Declaration of vaccine production facilities

1. Name of facility
MassBiologics

2. Location (Mailing Address)
University of Massachusetts Medical School
Boston, Massachusetts 02130

3. General description of the types of diseases covered:
Diphtheria and tetanus caused by *Corynebacterium diphtheriae* and *Clostridium tetani*.

Vaccines:
- Tetanus and Diphtheria Toxoids Adsorbed - [TDVAX]

Form G

Declaration of vaccine production facilities

1. Name of facility
MCM Vaccine Company/Sanofi Pasteur, Inc.

2. Location (Mailing Address)
1 Discovery Drive
Swiftwater, PA 18370

3. General description of the types of diseases covered:
Diphtheria, tetanus, pertussis, poliomyelitis, hepatitis B, and invasive disease due to *Haemophilus influenzae* type b.

Vaccines:

- Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus, Haemophilus b Conjugate [Meningococcal Protein Conjugate] and Hepatitis B [Recombinant] Vaccine - [VAXELIS]

Form G

Declaration of vaccine production facilities

1. Name of facility
Merck Sharp & Dohme Corp.

2. Location (Mailing Address)
PO Box 1000, UG2D-68
North Wales, Pennsylvania 19454

3. General description of the types of diseases covered:
Ebola virus disease, Invasive disease caused by *Haemophilus influenzae* type b; infection caused by all known subtypes of hepatitis B virus; Hepatitis A disease; cervical, vulvar and vaginal cancer and certain other diseases caused by Human Papillomavirus (HPV); Measles; Mumps; diseases caused by *Streptococcus pneumoniae*; Rotavirus disease; Rubella (German measles) disease; Varicella disease caused by the varicella-zoster virus (VZV); Herpes zoster (shingles) disease.

Vaccines:

- Ebola Zaire Vaccine, Live - [ERVEBO]
- Haemophilus b Conjugate Vaccine (Meningococcal Protein Conjugate) - [PedvaxHIB]
- Hepatitis A Vaccine, Inactivated - [VAQTA]
- Hepatitis B Vaccine (Recombinant) - [RECOMBIVAX HB]
- Human Papillomavirus Quadrivalent (Types 6, 11, 16, 18) Vaccine, Recombinant - [Gardasil]
- Human Papillomavirus 9-valent Vaccine, Recombinant - [Gardasil 9]
- Measles, Mumps, and Rubella Virus Vaccine, Live - [M-M-R II]
- Measles, Mumps, Rubella and Varicella Virus Vaccine Live - [ProQuad]
- Pneumococcal Vaccine, Polyvalent - [Pneumovax 23]
- Rotavirus Vaccine, Live, Oral, Pentavalent - [RotaTeq]
- Varicella Virus Vaccine Live - [Varivax]
- Zoster Vaccine, Live - [Zostavax]

Form G

Declaration of vaccine production facilities

1. Name of facility
Organon Teknika Corporation, LLC

2. Location (Mailing Address)
100 Rodolphe Street
Building 1300
Durham, North Carolina 27712

3. General description of the types of diseases covered:
For the prevention of tuberculosis

Vaccines:
- BCG Live - [BCG Vaccine]

Form G

Declaration of vaccine production facilities

1. Name of facility
Protein Sciences Corporation

2. Location (Mailing Address)
1000 Research Parkway
Meriden, Connecticut 06450-7159

3. General description of the types of diseases covered:
Disease caused by influenza virus subtypes A and B

Vaccines:
- Influenza Vaccine (Trivalent) - [Flubok]
- Influenza Vaccine (Quadrivalent) - [Flubok Quadrivalent]

Form G

Declaration of vaccine production facilities

1. Name of facility
Sanofi Pasteur, Inc.

2. Location (Mailing Address)
1 Discovery Drive
Swiftwater, PA 18370

3. General description of the types of diseases covered:
Dengue disease caused by dengue virus serotypes 1, 2, 3 and 4; influenza disease caused by pandemic (H1N1) 2009 virus; influenza disease caused by H5N1 subtype; influenza disease caused by influenza virus subtype A and type B; invasive meningococcal disease caused by Neisseria meningitidis serogroups A, C, Y and W-135; invasive meningococcal disease caused by Neisseria meningitidis serogroups A, C, Y, and W-135; and yellow fever acute viral illness caused by a mosquito-borne flavivirus

Vaccines:

- Dengue Tetravalent Vaccine, Live - [DENGVAXIA]
- Influenza A (H1N1) 2009 Monovalent Vaccine
- Influenza Virus Vaccine, H5N1 (for National Stockpile)
- Influenza Virus Vaccine (Trivalent, Types A and B) - [Fluzone, Fluzone High-Dose, and Fluzone Intradermal]
- Influenza Virus Vaccine (Quadrivalent, Types A and Types B) - [Fluzone Quadrivalent]
- Meningococcal (Groups A, C, Y and W-135) Polysaccharide Diphtheria Toxoid Conjugate Vaccine - [Menactra]
- Meningococcal Polysaccharide Vaccine, Groups A, C, Y and W-135 Combined - [Menomune-A/C/Y/W-135]
- Yellow Fever Vaccine - [YF-Vax]

Form G

Declaration of vaccine production facilities

1. Name of facility
Seqirus Inc.

(b)(5)

2. Location (Mailing Address)
Seqirus Inc.
475 Green Oaks Parkway
Holly Springs, NC 27540

3. General description of the types of diseases covered:
Influenza

Vaccines:
- Influenza A (H5N1) Monovalent Vaccine, Adjuvanted - [AUDENZ]
- Influenza Vaccine, Adjuvanted - [FLUAD and FLUAD QUADRIVALENT]
- Influenza Vaccine – [Flucelvax Quadrivalent]
- Influenza Virus Vaccine (Trivalent, Types A and B) - [Flucelvax]

Form G

Declaration of vaccine production facilities

1. Name of facility
Wyeth Pharmaceuticals, Inc

2. Location (Mailing Address)
Pfizer, Inc.,
401 N. Middletown Road
Pearl River, New York 10965

3. General description of the types of diseases covered:
Invasive disease caused by *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F and otitis media caused by S. pneumoniae serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, and invasive disease caused by *Neisseria meningitides* serogroup B.

Vaccines:
- Meningococcal Group B Vaccine - [TRUMENBA]
- Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM197 Protein) - [Prevnar 13]

Appendix A

Biological Select Agents and Toxins

Biological Select Agents and Toxins are biological pathogens and toxins that the United States has determined have the potential to pose a severe threat to public health and safety, animal and plant health, or animal and plant products. The possession, use, and transfer of these agents is regulated by the U.S. Department of Health and Human Services (HHS) Centers for Disease Control and Prevention and the U.S. Department of Agriculture Animal and Plant Health Inspection Service under the Select Agent Regulations found in Part 73 of Title 42 of the Code of Federal Regulations, Part 331 of Title 7 of the Code of Federal Regulations, and Part 121 of Title 9 of the Code of Federal Regulations. Information on Biological Select Agents and Toxins can be found on the National Select Agent Registry website: http://www.selectagents.gov.

HHS Select Agents and Toxins
Abrin
Bacillus cereus Biovar *anthracis*
Botulinum neurotoxins
Botulinum neurotoxin-producing species of *Clostridium*
Conotoxins (alpha)
Coxiella burnetii
Crimean-Congo haemorrhagic fever virus
Diacetoxyscirpenol
Eastern Equine Encephalitis virus
Ebola virus
Francisella tularensis
Lassa fever virus
Lujo virus
Marburg virus
Monkeypox virus
Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
Ricin
Rickettsia prowazekii
SARS-associated coronavirus (SARS-CoV)
Saxitoxin
South American Haemorrhagic Fever viruses: Chapare, Guanarito, Junin, Machupo, Sabia
Staphylococcal euterotoxius (A, B, C, D, E subtypes)
T-2 toxiu
Tetrodotoxin
Tick-borne encephalitis complex (flavi) viruses: Far Eastern Tick-borne encephalitis, Siberian subtype, Kyasanur Forest disease, Omsk Hemorrhagic Fever
Variola major virus (Smallpox virus)
Variola minor virus (Alastrim)
Yersinia pestis

OVERLAP Select Agents and Toxins
Bacillus anthracis
Bacillus anthracis Pasteur strain
Brucella abortus

Brucella melitensis
Brucella suis
Burkholderia mallei (formerly *Pseudomonas mallei*)
Burkholderia pseudomallei (formerly *Pseudomonas pseudomallei*)
Hendra virus
Nipah virus
Rift Valley fever virus
Venezuelan Equine Encephalitis virus

USDA Select Agents and Toxins
African horse sickness virus
African swine fever virus
Avian influenza virus (highly pathogenic)
Classical swine fever virus
Foot-and-mouth disease virus
Goat pox virus
Lumpy skin disease virus
Mycoplasma capricolum subspecies *capripneumoniae* (contagious caprine pleuropneumonia)
Mycoplasma mycoides subspecies mycoides small colony (*Mmm* SC) (contagious bovine pleuropneumonia)
Newcastle disease virus (virulent virus serotype1)
Peste des petits ruminants virus
Rinderpest virus
Sheep pox virus
Swine vesicular disease virus

USDA PLANT PROTECTION AND QUARANTINE (PPQ) Select Agents and Toxins
Coniothyrium glycines (formerly Phoma glycinicola and Pyrenochaeta glycines)
Peronosclerospora philippinensis (Peronosclerospora sacchari)
Ralstonia solanacearum
Rathayibacter toxicus
Sclerophthora rayssiae
Synchytrium endobioticum
Xanthomonas oryzae

Appendix A

NIAID Category A, B, and C Priority Pathogens

The National Institute of Allergy and Infectious Disease (NIAID) categorization of pathogens identifies specific pathogens as priorities for additional research efforts as part of the NIAID biodefense research agenda.

Additional information on NIAID Category A, B, and C Priority Pathogens is available at: https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens

Category A pathogens are those organisms/biological agents that pose the highest risk to national security and public health because they

- Can be easily disseminated or transmitted from person to person
- Result in high mortality rates and have the potential for major public health impact
- Might cause public panic and social disruption
- Require special action for public health preparedness

Category A Priority Pathogens

Bacillus anthracis (anthrax)
Clostridium botulinum toxin (botulism)
Yersinia pestis (plague)
Variola major (smallpox) and other related pox viruses
Francisella tularensis (tularemia)
Viral hemorrhagic fevers: Arenaviruses (Junin virus, Machupo virus, Guanarito virus, Chapare virus, Lassa virus, and Lujo virus); Bunyaviruses (Hantaviruses, Rift Valley Fever virus, Crimean Congo Hemorrhagic Fever virus); Flaviruses (Dengue virus); Filoviruses (Ebola, Marburg viruses)

Category B pathogens are the second highest priority organisms/biological agents. They

- Are moderately easy to disseminate
- Result in moderate morbidity rates and low mortality rates
- Require specific enhancements for diagnostic capacity and enhanced disease surveillance

Category B Priority Pathogens

Burkholderia pseudomallei (melioidosis)
Coxiella burnetii (Q fever)
Brucella species (brucellosis)
Burkholderia mallei (glanders)
Chlamydia psittaci (Psittacosis)
Ricin toxin (*Ricinus communis*)
Epsilon toxin (*Clostridium perfringens*)
Staphylococcus enterotoxin B (SEB)
Typhus fever (*Rickettsia prowazekii*)
Food- and Waterborne Pathogens

- Bacteria: Diarrheagenic *E.coli*, Pathogenic Vibrios, *Shigella* species, Salmonella, *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*
- Viruses: Caliciviruses, Hepatitis A virus
- Protozoa: *Cryptosporidium parvum*, *Cyclospora cayatanensis*, *Giardia lamblia*, *Entamoeba histolytica*, *Toxoplasma gondii*, *Naegleria fowleri*, *Balamuthia mandrillaris*
- Fungi: Microsporidia

Mosquito-born viruses: West Nile Virus, LaCrosse encephalitis virus, California encephalitis virus, Venezuelan equine encephalitis virus, Eastern equine encephalitis virus, Western equine encephalitis virus, Japanese encephalitis virus, St. Louis encephalitis virus, Yellow fever virus, Chikungunya virus, Zika virus

Category C pathogens are the third highest priority and include emerging pathogens that could be engineered for mass dissemination in the future because of

- Availability
- Ease of production and dissemination
- Potential for high morbidity and mortality rates and major health impact

Category C Priority Pathogens

Emerging infectious disease threats such as Nipah virus, Hendra virus, and additional hantaviruses
Tickborne hemorrhagic fever viruses such as Bunyaviruses (Severe Fever with Thrombocytopenia Syndrome virus, Heartland virus) and Flaviviruses (Omsk Hemorrhagic Fever virus, Alkhurma virus, Kyasanur Forest virus)
Tickborne encephalitis complex flaviviruses (Tickborn encephalitis virus, European subtype, Far Eastern subtype, Siberian subtype, Powassan/Deer Tick virus)
Tuberculosis, including drug-resistant TB
Influenza virus
Other Rickettsias
Rabies virus
Prions
Coccidioides spp.
Severe acute respiratory syndrome associated coronavirus (SARS-CoV), MERS-CoV, and other highly pathogenic human corona viruses
Antimicrobial resistance, excluding research on sexually transmitted organisms, unless the the resistance is newly emerging*

- Research on mechanisms of antimicrobial resistance
- Studies of the emergence and/or spread of antimicrobial resistance genes within pathogen populations
- Studies of the emergence and/or spread of antimicrobial-resistant pathogens in human populations
- Research on therapeutic approaches that target resistance mechanisms
- Modification of existing antimicrobials to overcome emergent resistance

Antimicrobial research, as related to engineered threats and naturally occurring drug-resistant pathogens, focused on development of broad-spectrum antimicrobials
Immunology studies that advance our understanding of host defenses applicable to the biodefense effort, for example: Adjuvants, Innate Immunity, Adaptive Immunity, Mucosal Immunity
Additional Emerging Infectious Diseases/Pathogens: Acanthamebiasis, Anaplasmosis, Australian bat lyssavirus, *Babesia*, atypical, *Bartonella henselae*, BK virus, *Bordetella pertussis*, *Borrelia mayonii*, *Borrelia miyamotoi*, Ehrlichiosis, Enterovirus 68, Enterovirus 71, Hepatitis C, Hepatitis E, Human herpesvirus 6, Human herpesvirus 8, JC virus, Leptospirosis, Mucormycosis, Poliovirus, Rubeola (measles), *Streptococcus* Group A

* NIAID Category C Antimicrobial Resistance—Sexually Transmitted Excluded Organisms: Bacterial vaginosis, *Chlamydia trachomatis*, Cytomegalovirus, *Granuloma inguinale*, *Hemophilus ducreyi*, Hepatitis B virus, Hepatitis C virus, Herpes Simplex virus, Human immunodeficiency virus, Human papillomavirus, *Treponema pallidum*, *Trichomonas vaginalis*

Appendix B

Compiled list of microorganisms and toxins used for biodefense research

MICROORGANISM	CATEGORY
African horse sickness virus	USDA Select Agent
African swine fever virus	USDA Select Agent
Avian influenza virus (highly pathogenic)	USDA Select Agent
Bacillus anthracis	Overlap Select Agent + NIAID Category A
Bacillus anthracis Pasteur strain	Overlap Select Agent
Bacillus cereus Biovar anthracis	HHS Select Agent
Brucella abortus	Overlap Select Agent
Brucella melitensis	Overlap Select Agent
Brucella suis	Overlap Select Agent
Burkholderia mallei	Overlap Select Agent
Burkholderia pseudomallei	Overlap Select Agent
Chapare virus	HHS Select Agent
Classical swine fever virus	USDA Select Agent
Clostridium species producing botulinum neurotoxin	HHS Select Agent + NIAID Category A
Coniothyrium glycines	PPQ Select Agent
Coxiella burnetti	HHS Select Agent
Crimean-Congo hemorrhagic fever virus	HHS Select Agent
Dengue virus	NIAID Category A
Eastern equine encephalitis virus	HHS Select Agent
Ebola virus	HHS Select Agent + NIAID Category A
Foot-and-mouth disease virus	USDA Select Agent
Francisella tularensis	HHS Select Agent + NIAID Category A
Goatpox virus	USDA Select Agent
Guanarito virus	HHS Select Agent + NIAID Category A
Hantaviruses	NIAID Category A
Hendra virus	Overlap Select Agent
Influenza A virus, reconstructed replication-competent pandemic 1918 strains	HHS Select Agent
Junin virus	HHS Select Agent + NIAID Category A
Kyasanur Forest disease virus	HHS Select Agent
Lassa virus	HHS Select Agent + NIAID Category A
Lujo virus	HHS Select Agent
Lumpy skin disease virus	USDA Select Agent
Lymphocytic choriomeningitis virus	NIAID Category A
Machupo virus	HHS Select Agent + NIAID Category A
Marburg virus	HHS Select Agent + NIAID Category A
Monkeypox virus	HHS Select Agent
Mycoplasma mycoides	USDA Select Agent
Newcastle disease virus	USDA Select Agent
Nipah virus	Overlap Select Agent
Omsk hemorrhagic fever virus	HHS Select Agent
Rathayibacter toxicus	PPQ Select Agent

Rickettsia prowazekii	HHS Select Agent
Rift Valley fever virus	Overlap Select Agent + NIAID Category A
Sabia virus	HHS Select Agent
Severe acute respiratory syndrome-related coronavirus	HHS Select Agent
Tick-borne encephalitis complex flavivirus, Far Eastern subtype	HHS Select Agent
Tick-borne encephalitis complex flavivirus, Siberian subtype	HHS Select Agent
Variola major virus	HHS Select Agent + NIAID Category A
Variola minor virus	HHS Select Agent
Venezuelan equine encephalitis virus	Overlap Select Agent
Yersinia pestis	HHS Select Agent + NIAID Category A
TOXINS	**CATEGORY**
Abrin	HHS Select Toxin
Alpha conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence X1CCX2PACGX3X4X5X6CX7)	HHS Select Toxin
Botulinum neurotoxins	HHS Select Toxin
Diacetoxyscirpenol	HHS Select Toxin
Ricin	HHS Select Toxin
Saxitoxin	HHS Select Toxin
Staphylococcal enterotoxins A, B, C, D, E subtypes	HHS Select Toxin
T-2 toxin	HHS Select Toxin
Tetrodotoxin	HHS Select Toxin