IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF PUERTO RICO

TROPICAL CHILL CORP., et al., Plaintiffs, v. PIERLUISI URRUTIA, et al., Defendants.	CIVIL NO.: 21-1411 (RAM-MEL)

REPORT & RECOMMENDATION

Plaintiffs Tropical Chill Corp. ("Tropical Chill"), Jasmín Vega González ("Ms. Vega"), Eliza Llenza ("Ms. Llenza"), and René Matos Ruiz ("Mr. Matos") bring suit pursuant to 42 U.S.C. § 1983, 28 U.S.C. §§ 1331, 1343(a), 2201, and 2202 against the Honorable Pedro R. Pierluisi Urrutia ("the Governor"), in his official capacity as Governor of the Commonwealth of Puerto Rico, and against Carlos R. Mellado López in his official capacity as Secretary of Health of the Commonwealth of Puerto Rico (collectively "Defendants"). ECF No. 7 at 1; ECF No. 35 at 1, 8. Specifically, Plaintiffs challenge Executive Order No. 2021-075 ("EO75") and Regulation of the Secretary of Health No. 138-A ("Regulation 138-A"). Plaintiffs contend that EO75 and Regulation 138-A violate their substantive due process rights under the Fourteenth Amendment of the United States Constitution. Ms. Vega also argues that EO75 violates her rights under the Religious Freedom Restoration Act ("RFRA"), 42 U.S.C. § 2000bb-§ 3 2000bb. Finally, Plaintiffs invoke the court's supplementary jurisdiction to allege that EO75 and Regulation 138-A violate the Puerto Rico Constitution. Plaintiffs amended the complaint, filed a Rule 15(d) supplemental pleading, and moved for a preliminary injunction to prevent the implementation and enforcement of EO75 and Regulation 138-A—the subject of this report and

recommendation. ECF No. 35; ECF No. 67; ECF No. 7. Defendants have opposed the request for preliminary injunction. ECF No. 20. The court held a preliminary injunction hearing during which Plaintiffs and Defendants presented evidence and arguments. After considering the arguments of the parties, the pertinent authorities, and the evidence produced at the evidentiary hearing, Plaintiffs' Motion for Preliminary Injunction should be denied.

I. EXECUTIVE ORDER 75 & HEALTH REGULATION 138-A

The challenged government mandates were promulgated by the government of Puerto Rico in response to the SARS-CoV-2 coronavirus pandemic, an outbreak more commonly known by the name of the disease caused by the coronavirus—the COVID-19 pandemic.

A. Regulation 138-A

On August 5, 2021, the Puerto Rico Department of Health issued Regulation 138-A, an amendment of Health Regulation 138, which requires proof of COVID-19 vaccination as "an essential document for a doctor to issue a health certificate." ECF No. 35-1 at 2. A health certificate is required to work in many different occupations in Puerto Rico. Regulation 138-A provides for a medical exemption for those cases where "the patient has a compromised immune system or there is a medical contraindication that prevents inoculation. This must be certified by a doctor authorized to practice in Puerto Rico or by the doctor who issues the health certificate." ECF No. 35-1 at 3. If a medical contraindication is temporary, a doctor must so certify, and the person is required to comply with the vaccination requirement to be issued subsequent certificates. ECF No. 35-1 at 3. Regulation 138-A also provides a religious exemption whereby a person may show a sworn statement to the issuing doctor "in accordance with the Executive Orders in force" proving that the "vaccine goes against the dogmas of the patient's religion." ECF No. 35-1 at 3. There are no other exemptions to the COVID-19 vaccination requirement

under Regulation 138-A. Regulation 138-A remains in effect at the time of this report and recommendation.

B. Executive Order 075

In August 2021, the Governor of Puerto Rico issued Executive Orders 2021-062, 2021-063, and 2021-064. ECF Nos. 35-2; 35-5; 35-6. On November 15, 2021, the Governor issued Executive Order 2021-075 which consolidated the existing COVID-19 executive orders and expressly repealed executive orders 062, 063, and 064. Joint Exhibit I at 32. EO75 is the executive order challenged in this case.

Section 6 of EO75 requires employees and contractors who work in public agencies of the Commonwealth of Puerto Rico, and contractors and their employees who frequently visit government offices to either (1) supply proof to their employers of being "fully vaccinated" against COVID-19; (2) be tested every 7 days for COVID-19; or (3) to furnish their employers a positive COVID-19 test result performed within the last 3 months along with a letter from a healthcare provider or government official certifying they have recovered. Joint Exhibit I at 17. For these government employees and contractors, Section 6 does not require them to furnish "the documents associated with a medical or religious exception" to comply with the second or third option. Joint Exhibit I at 17.

Section 9 of EO75 establishes that private sector employees and persons working at "hotels, . . . lodgings, restaurants (including fast foods, food courts, and cafeterias)[,] . . . small cafeterias, . . . [and] supermarkets" must (1) show proof to their employer of being "fully vaccinated" against COVID-19; (2) be tested every 7 days for COVID-19; or (3) to furnish their employer a positive COVID-19 test result performed within the last 3 months and evidence that they have recovered. Joint Exhibit I at 25. In addition, Section 9 commands that any business

that has 50 or more employees require that its employees comply with one of the same three conditions. Joint Exhibit I at 25. Section 9 makes "[e]very employer, merchant, owner, manager, or similar person" responsible for checking that employees in their business are complying with EO75. Joint Exhibit I at 26. No exemptions for any reason are listed.

Section 10 establishes that "restaurants (including fast foods [*sic*], food courts, and cafeterias)[,] bars, "chinchorros," small cafeterias, sports bars, theaters, movie theaters, stadiums, convention and activity centers that sell alcoholic beverages or prepared food, hotels, *paradores*, lodgings, beauty salons, barber shops, aesthetics salon [*sic*], spas, gyms, and casinos" are required to check that all visitors to their businesses comply with one of three requirements. Joint Exhibit I at 27. The visitor must either (1) show proof of being "fully vaccinated" against COVID-19; (2) furnish a negative COVID-19 test performed within the last 72 hours prior to the visit; or show a positive COVID-19 test result performed within the last three months accompanied with documents evidencing the visitor's recovery and ability to be in a public space. Any business covered in Section 10 that prefers not to check the vaccination or COVID-19 test status of their customers is required to "limit the capacity of the business to 50%, in accordance with the building code in effect." Joint Exhibit I at 29. Section 10 exempts children younger than the age of five who cannot yet be vaccinated until January 31, 2022 "given that the vaccination process for them is under way." Joint Exhibit I at 28. No exemptions for any other reason are listed for either visitors to or the owners of the businesses falling under Section 10.

Section 13 mandates that the Department of Health continue to facilitate testing to detect the virus. Section 13 also requires that the Department of Health publish "in electronic media, including on the webpage of the Department of Health, the locations where testing is being conducted." Joint Exhibit I at 30.

Finally, as relevant to this case, Section 14 establishes the penalties for non-compliance with EO75. Any person or business who fails to comply with EO75 is subject to criminal prosecution under 25 L.P.R.A. § 3654 and subject to a penalty of a term of imprisonment not more than 6 months, or a fine of not more than $5,000, or both. Joint Exhibit I at 30. Section 14 also states that the same non-complying person could also be subject to Section 33 of the Organic Act of the Department of Health, and subject to conviction for a misdemeanor carrying the penalty of up to six months imprisonment and up to a $5,000 fine. Joint Exhibit I at 30. Additionally, for anyone who fails to comply with EO75 for a second time within the term of one year, "the fine imposed may be raised to a maximum of ten thousand dollars ($10,000)." Joint Exhibit I at 31.

II. PROCEDURAL BACKGROUND

On August 27, 2021 Plaintiffs initiated this suit by filing a "Complaint for Declaratory and Injunctive Relief." ECF No. 1. Four days later, on August 31, 2021, Plaintiffs filed the instant "Motion for Preliminary Injunction and Hearing" to which Defendants responded in opposition on September 16, 2021. ECF No. 7; ECF No. 20. Plaintiffs filed a "Reply to Opposition to Motion for Preliminary Injunction" on October 4, 2021 to which Defendants surreplied on October 15, 2021. ECF No. 29; ECF No. 42. Plaintiffs filed the first "Amended Complaint for Declaratory and Injunctive Relief" on October 7, 2021. ECF No. 35; ECF Nos. 40, 41. On November 23, 2021, following the issuance of Executive Order 075, Plaintiffs filed a "Rule 15(d) Supplemental Pleading." ECF No. 67.

On December 6, 7, 8, 9, 13, and 14, 2021, the court held an evidentiary hearing during which both parties were provided an opportunity to present evidence. In addition to the testimony of all four Plaintiffs, Plaintiffs also presented the testimony of four expert witnesses,

one lay witness, and introduced 65 exhibits.[1] During their presentation of the evidence Defendants introduced the testimony of three expert witnesses and eight exhibits. The parties also introduced three joint exhibits.[2] On the last day of the evidentiary hearing, Plaintiffs filed a "Motion Requesting Order for Judicial Notice" in which they requested the court take judicial notice of 18 items. ECF No. 83. Defendants opposed Plaintiffs' motion for judicial notice. ECF No. 89. On December 27, 2021, Plaintiffs tendered a "Second Rule 15(d) Supplemental Pleading"; ECF No. 95; ECF No. 97. The court has not yet ruled on whether to allow a second supplemental pleading. ECF No. 98.[3]

III. FACTUAL BACKGROUND & CLAIMS

Plaintiffs specifically challenge two parts of the government mandates. First, they challenge the requirements under EO75 for private businesses to check the vaccination or COVID-19 test status of patrons. ECF No. 35 at 2; ECF No. 67 at 1–2. Second, Plaintiffs challenge the requirement of proof of COVID-19 vaccination to obtain a health certificate under Regulation 138-A. ECF No. 35 at 2. The circumstances as to each Plaintiff are as follows.

A. Tropical Chill Corp.

Plaintiff Tropical Chill operates three ice cream stores, with one location in San Juan, a second location in Guaynabo, and a third location in Dorado, Puerto Rico. ECF No. 27 at 35. Tropical Chill's San Juan location is 1500 square feet and operates a drive through, the Guaynabo location is 1400 square feet, and the Dorado location is 400 square feet located in the

[1] The exhibits referenced in this report and recommendation have kept the same identification numbers and letters used during the evidentiary hearing.
[2] In the days following the evidentiary hearing, Plaintiffs and Defendants submitted certified translations of exhibits that were introduced in the Spanish language. ECF Nos. 86, 88.
[3] Since the preliminary injunction hearing, the Governor has issued new or additional Executive Orders regarding the COVID-19 pandemic, the certified translation of which has not been submitted to the court. The Plaintiffs' tendered "Second Rule 15(d) Supplemental Pleading" aims to address these new mandates. ECF No. 95-1. However, inevitably the fast-paced developments of the pandemic and the continuously changing executive orders present a constantly moving target for judicial review as new circumstances develop.

food court area of a shopping center. The owner of Tropical Chill, Jaime Vega, who testified at the preliminary injunction hearing on December 6, 2021, is a strong believer in the efficacy of vaccinations and his entire family is vaccinated against COVID-19. However, according to Mr. Vega, the vaccine checks required of restaurants by EO75 severely inhibits the business model of Tropical Chill and has resulted in unsustainable financial losses for the corporation.

The mission statement of Tropical Chill is to "sell[] ten minutes of happiness to our customers" through serving ice cream in a family environment. Hearing, Dec. 6, at 9:23 AM. When presented with the option of checking vaccination status or operating at 50 percent capacity, Mr. Vega decided that inquiring into the vaccination status of his customers was uncomfortable, improper, and undermined the happy experience Tropical Chill seeks to provide. Mr. Vega also conveyed that Tropical Chill's employees were not comfortable asking about the vaccination status of customers. Mr. Vega explained that the requirement also put his employees in the difficult position of trying to determine the true age of a child to know whether EO75 would require that child to be vaccinated to enter the store.

Secondly, Mr. Vega explained that as a small-business owner he "run[s] a very tight ship," meaning that he must keep costs low. Hearing, Dec. 6, 9:42 AM. For example, to keep costs down, Mr. Vega personally acts as a plumber, a handyman, or in any other role needed at Tropical Chill's three stores. Hearing, Dec. 6, at 9:42 AM. As a matter of expense, Mr. Vega testified that he did not believe it would be financially feasible for him to hire an extra employee dedicated to check the vaccination status of patrons or to take orders outside the store because that practice would not result in fewer losses than what he is incurring by operating at 50 percent capacity. Hearing, Dec. 6, at 10:17–10:18 AM. However, Mr. Vega has not attempted any such procedure because he did not want to "bend [his] beliefs to get more customers." Hearing, Dec.

6, at 10:17–10:18 AM. Mr. Vega acknowledged that he was not sure whether customers had been turned away because any store had reached its full 50 percent capacity. Mr. Vega explained that the stores only log transactions rather than the number of visitors. However, he testified that the San Juan store, which includes the option of a drive through, has been the store that has incurred the biggest losses in sales. Hearing, Dec. 6, at 10:19 AM

For the above reasons, Mr. Vega decided that Tropical Chill would only operate at 50 percent capacity rather than check the vaccination status of its customers. Accordingly, since mid-August 2021 when the vaccine check requirement was announced, revenue from Tropical Chill's three stores decreased significantly. Mr. Vega reported that Tropical Chill's revenue in the three months preceding the mandate totaled $235,000, while the revenue for the three months after the mandate was $190,000. Hearing, Dec. 6, at 9:30 AM. This change amounted to lost revenue of "almost 20 percent for those months" after the executive order was issued. Hearing, Dec. 6, at 9:30 AM. Mr. Vega dismissed seasonality as a cause for the decrease testifying that in the 13 years he has operated Tropical Chill, seasonality led to a decrease in sales of only about two to four percent. Hearing, Dec. 6, at 9:30 AM. As a result, Mr. Vega stated that Tropical Chill is operating at a loss, Mr. Vega has not collected a salary in the month, and the stores have cut employee hours. Mr. Vega estimated that Tropical Chill could continue to operate under the current restrictions for 30 to 60 days before the losses would result in employee layoffs.

As such, Tropical Chill alleges that EO75 infringes upon Tropical Chill's right to earn a living and use its property as it sees fit, without sufficient government justification for restricting or infringing on those rights. ECF No. 35 at 29. Tropical Chill also claims that EO75 compels it to violate its customers' constitutional right to privacy. ECF No. 35 at 32.

B. Jasmín Vega González

Plaintiff Jasmín Vega owns and operates a short-term rental property through the platform Airbnb near Mayagüez, Puerto Rico. The property is called "Hillside Cabin" and is located on a mountain in the countryside. The cabin itself is located 200 feet from the entrance of the property and the closest neighbor to the structure is 220 feet away. The property has a maximum capacity of four people. Security cameras are located at the entrance and parking area to monitor ingress and egress to the property. Ms. Vega testified at the preliminary injunction hearing that for guests to make a reservation at the cabin they must provide personal information confirming that all the guests are relatives. Ms. Vega stated that she does not permit parties or events on the property. However, guests of Hillside Cabin are free to come and go to the property and visit local attractions. According to Ms. Vega, she never has personal contact with any of the guests.

Ms. Vega explained that because of her religious convictions she does not believe in vaccinations and has refused to be vaccinated for any reason since the age of seventeen. She is opposed, on account of her faith, to all mandates requiring her to check for proof of vaccination or to ask for a negative COVID-19 test result. Her "personal and religious interpretation is that we are reaching the last days" and these requirements, she explained, are part of the "mark of the Beast" described in Chapter 13 of the Book of Revelation of the Bible. Hearing, Dec. 7, at 2:27 PM. Ms. Vega objects to participating in the checks because her compliance makes her an "accomplice of everything." Hearing, Dec. 7, at 1:49–1:52 PM. According to Ms. Vega, her own personal refusal to be vaccinated results in her feeling marginalized, and reminds her of "back in the day, as the Bible says, some had to run, flee, hide, and whoever believed in God and spoke about God was killed." Hearing, Dec. 7, at 1:56–1:57 PM.

Ms. Vega testified briefly as to the impact she believes EO75 is having on Hillside Cabin. Ms. Vega has only operated Hillside Cabin since December 2020. Before the executive order requiring vaccine checks or proof of a negative COVID-19 test were required, Ms. Vega reported that for several months Hillside Cabin had a fully booked occupancy for "two or three months in a row." Hearing, Dec. 7, at 2:41 PM. After the issuance of the executive order, her property is usually occupied about 20 nights a month. Hearing, Dec. 7, at 2:09 PM; 2:41 PM. However, when asked whether as the owner she was actively checking whether her guests were vaccinated or had a negative COVID-19 test to stay at Hillside Cabin, she refused to answer, invoking the Fifth Amendment. Hearing, Dec. 7, at 2:28 PM.

Ms. Vega claims that EO75 deprives her of her right to earn an honest living, without sufficient justification for restricting or infringing on her rights. ECF No. 35 at 32. Second, she alleges that EO75 compels her to violate her guests' constitutional right to privacy. ECF No. 35 at 32. Third, Ms. Vega claims that EO75 burdens her constitutional rights to privacy, personal autonomy, bodily integrity, and medical choice because she must be vaccinated or submit to a COVID-19 test whenever she wants to go to a restaurant or participate in other activities. ECF No. 35 at 32. Finally, Ms. Vega brings a claim under the Religious Freedom Restoration Act, claiming that EO75 violates her religious beliefs by obligating her to verify vaccination status, thereby leading her "to participate in and condone forced vaccination." ECF No. 35 at 36.

C. Eliza Llenza

Plaintiff Eliza Llenza is currently unemployed but is seeking employment. Ms. Llenza contracted and tested positive for COVID-19 in December 2020. Exhibit 42. On June 25 and September 17, 2021 Ms. Llenza presented lab tests which showed she still had antibodies for the SARS-CoV-2 virus. Exhibit 43, 44. Ms. Llenza believes these antibodies give her natural

immunity from the COVID-19 virus, and so has declined to be vaccinated. However, Ms. Llenza acknowledged that she has not consulted with a doctor about whether she should be vaccinated, nor has any doctor advised her not to be vaccinated. Ultimately she stated on cross-examination that her decision to be unvaccinated was "a personal decision of mine based on my lifelong experience. I know as a fact that when you get a virus of some sort you develop immunity." Hearing, Dec. 8, at 10:18 AM.

Ms. Llenza last worked as a field inspector for a private contractor selected to participate in a local disaster relief program in Puerto Rico after Hurricane María. Since her date of last employment, Ms. Llenza received training in "Planning for Disaster Debris Management" from the Federal Emergency Management Agency (FEMA) and in "Mold Clean-Up and Safety after Disasters" from Ana G. Méndez University. Exhibits 39, 40. However, Ms. Llenza believes that she can no longer work in disaster recovery for which she has been trained because EO75 requires her to be vaccinated.

Ms. Llenza was also certified as a "Professional Food Manager" in December 2019. Exhibit 41. She, however, does not have any experience in food management. Ms. Llenza also explained that positions in food preparation are no longer available to her because they would require she be vaccinated to obtain a health certificate, which she does not currently have.

Ms. Llenza sought a health certificate in 2021 after hearing that jobs were available in the food industry, such as at hotels and restaurants. Accordingly, Ms. Llenza began the health certification process with a doctor's office in August 2021 but testified that the office manager told Ms. Llenza that she would not be able to finish the health certification process unless she could present evidence of COVID-19 vaccination. Finally, Ms. Llenza also has a degree in

medical billing, but she does not believe she could get a job in a doctor's office because such a position would require that she be vaccinated and have a health certificate.

Ms. Llenza described that the challenged vaccine mandates have also impacted her ability to look for work and has impacted her personal life. Ms. Llenza discussed how the mandates have made it difficult for her to attend job fairs or interviews unless she can plan far enough ahead to get a test. She also testified how the requirement for vaccination or testing prevents her from seeing her children play in sports events, attend art exhibits, or even go to outdoor festivals. Ms. Llenza lamented that "she is treated practically as a criminal" because she is stopped at entrances when before she has never had to have anyone stop her anywhere.

For the above reasons, Ms. Llenza claims that EO75 and Regulation 138-A prevent her from obtaining employment in the fields for which she is trained, namely because to work in government or as a government contractor she is required by EO75 to be vaccinated, and because vaccination is required by Regulation 138-A to obtain a health certificate to work as a Professional Food Manager. ECF No. 35 at 29–30. Likewise, Ms. Llenza alleges that EO75 burdens her constitutional rights to privacy, personal autonomy, bodily integrity, and medical choice because she must be vaccinated or submit to a COVID-19 test whenever she wishes to "attend restaurants, bars, get a haircut, or stay in a hotel or Airbnb, among other activities." ECF No. 35 at 32.

D. René Matos Ruíz

Plaintiff René Matos Ruíz is a resident of San Germán, Puerto Rico, but works as a warehouse assistant and shelf attendant for Econo Supermarkets in Mayagüez, Puerto Rico. Mr. Matos is not currently vaccinated for COVID-19 and is opposed to vaccinations "based on [his] religious beliefs and convictions, and also [his] knowledge and the searches that I

conducted of the ingredients and compounds in the product." Hearing, Dec. 7, at 3:16–3:17 PM.[4] Consequently, Mr. Matos, a 58-year-old man, has not been vaccinated since he was a child. However, Econo Supermarkets requires Mr. Matos to renew his health certificate annually in order to work. Despite not being vaccinated, Mr. Matos currently possesses a health certificate and is working. Mr. Matos hurried to renew his certificate on August 6, 2021, the day after Regulation 138-A was issued, fearing that he would be denied a health certificate because of his non-vaccinated status. Despite not having proof of COVID-19 vaccination, Mr. Matos was granted a health certificate. Mr. Matos is concerned that by the next time he must renew his health certificate, in August 2022, he will be denied a certificate because he is not vaccinated, and he will therefore lose his job.

Mr. Matos is not required to be vaccinated by Econo Supermarkets as long as he gets a weekly COVID-19 test. It is Mr. Matos' opinion that Econo Supermarkets' requirement is a result of the mandates because "they have mentioned that" their policy is in compliance with the law. Hearing, Dec. 7 at 5:10 PM. Accordingly, Mr. Matos and his supervisor reached a verbal agreement whereby Mr. Matos will be given Thursdays off, during which he can complete his weekly COVID-19 test.

Mr. Matos also explained the impact that EO75 has had on his personal life. He described how not having a vaccine card has meant that he will be turned away from certain restaurants and he has had difficulty attending even public festivals such as those held by the local community in San Germán. He also testified that fixing his day off on a Thursday takes him out

[4] Mr. Matos's religious basis for objecting to vaccination is unclear, and he does not join Ms. Vega's RFRA claim or bring his own. When asked about the specifics of his religious convictions, Mr. Matos responded that he did not belong to any organized religion, nor does he have any religious preference. On cross examination he listed his three religious convictions: "Number one: I believe in God. Number two: I believe in preserving your . . . sound and healthy body. Number three: Obeying the Word." Hearing, Dec. 7, at 4:52–4:53 PM. However, Mr. Matos acknowledged that vaccination does not go against any religious "dogma" that he holds. Hearing, Dec. 7 at 5:15 PM.

of the normal rotation of days, and he would prefer to have his vacation days return to a normal rotation. He would also prefer not to interrupt one of his days off to be tested.

For the above reasons, Mr. Matos claims that Regulation 138-A deprives him of his right to earn an honest living and threatens his property interest in his health certificate without sufficient justification for restricting or infringing upon those rights. ECF No. 35 at 31. Mr. Matos also claims that the EO75 burdens his constitutional rights to privacy, personal autonomy, bodily integrity, and medical choice because he must either be vaccinated or submit to COVID-19 testing whenever he wants to go to "restaurants, bars, get a haircut, stay in a hotel or Air[bnb], among other activities." ECF No. 35 at 32.

IV. DISCUSSION

"A preliminary injunction is an extraordinary and drastic remedy, one that should not be granted unless the movant, *by a clear showing*, carries the burden of persuasion." Mazurek v. Armstrong, 520 U.S. 968, 972 (1997) (citation omitted) (emphasis in original). The movant bears the burden of persuading the district court that the following four elements are satisfied: "(1) a likelihood of success on the merits, (2) a likelihood of irreparable harm absent interim relief, (3) a balance of equities in the plaintiff's favor, and (4) service of the public interest." Arborjet, Inc. v. Rainbow Treecare Sci. Advancements, Inc., 794 F.3d 168, 171 (1st Cir. 2015). Here, Plaintiffs, as the movants seeking injunctive relief, bear the burden of establishing that the above factors weigh in their favor.

The first two factors in the preliminary injunction analysis are the most important—likelihood of success and irreparable harm. Charlesbank Equity Fund II v. Blinds To Go, Inc., 370 F.3d 151, 162 (1st Cir. 2004). Regarding likelihood of success, "the district court is required only to make an estimation of likelihood of success and 'need not predict the eventual outcome

on the merits with absolute assurance.'" Corp. Techs., Inc. v. Harnett, 731 F.3d 6, 10 (1st Cir. 2013) (quoting Ross-Simons of Warwick, Inc. v. Baccarat, Inc., 102 F.3d 12, 16 (1st Cir. 1996)). If the moving party fails to show it is likely to succeed on the merits, a court may act within its discretion to deny relief without addressing the remaining three factors. New Comm. Wireless Servs., 287 F.3d at 9. Therefore, "[l]ikelihood of success is the main bearing wall of the four-factor framework." Ross–Simons, 102 F.3d at 16.

Nevertheless, "[h]ow strong a claim on the merits is enough depends on the balance of the harms: the more net harm an injunction can prevent, the weaker the plaintiff's claim on the merits can be while still supporting some preliminary relief." Hoosier Energy Rural Elec. Coop., Inc. v. John Hancock Life Ins. Co., 582 F.3d 721, 725 (7th Cir. 2009). "The burden of demonstrating that a denial of interim relief is likely to cause irreparable harm rests squarely upon the movant" and the potential for irreparable injury "must not be assumed, it must be demonstrated . . . ." Charlesbank Equity Fund II, 370 F.3d at 162; Narragansett Indian Tribe v. Guilbert, 934 F.2d 4, 5 (1st Cir. 1991). Like the required showing of the likelihood of success, "the measure of irreparable harm is not a rigid one; it has been referred to as a sliding scale, working in conjunction with a moving party's likelihood of success on the merits." Vaquería Tres Monjitas, Inc. v. Irizarry, 587 F.3d 464, 485 (1st Cir. 2009) (citations omitted).

A. Likelihood of Success on the Merits

a. Standing

Under Article III of the United States Constitution, the jurisdiction of Federal Courts is limited to "Cases" and "Controversies." Lujan v. Defenders of Wildlife, 504 U.S. 555, 559 (1992). This so being, as a threshold matter, federal courts must be satisfied that a plaintiff is the correct person to bring the case at bar by possessing the requisite constitutional standing. Id. at

560. Neither the Supreme Court nor the First Circuit has yet clarified what burden a plaintiff carries to demonstrate standing at the preliminary injunction stage; however, "several circuit courts have held that the 'merits' on which plaintiff must show a likelihood of success encompass not only substantive theories but also establishment of jurisdiction, including standing." Pietrangelo v. Sununu, 2021 WL 1254560 (D.N.H. Apr. 5, 2021) (citing Waskul v. Washtenaw Cnty. Cmty. Mental Health, 900 F.3d 250, 256 n.4 (6th Cir. 2018); Yazzie v. Hobbs, 977 F.3d 964, 966 (9th Cir. 2020)) (internal quotations omitted). "This is so because an affirmative burden of showing a likelihood of success on the merits . . . necessarily includes a likelihood of the court's reaching the merits, which in turn depends on a likelihood that plaintiff has standing." Pietrangelo, 2021 WL 1254560 (D.N.H. Apr. 5, 2021) (citing Waskul, 900 F.3d at 256 n. 4) (internal quotations omitted). Therefore, plaintiffs who fail to affirmatively produce evidence showing they possess a "substantial likelihood of standing" should not be granted a preliminary injunction on those claims. Food & Water Watch, Inc. v. Vilsack, 808 F.3d 905, 913 (D.C. Cir. 2015); New York v. United States Dep't of Homeland Sec., 969 F.3d 42, 59 (2d Cir. 2020). On specific claims discussed below, Plaintiffs have failed to show a substantial likelihood that they possess standing to pursue those claims, which weighs against their likelihood of success on the merits.

1. Ms. Vega's Standing for Her Economic Liberty and Property Rights Claim

Ms. Vega asserts that the vaccine or COVID-19 test check requirements to which her Airbnb is subject deprives her of her right to earn an honest living, in violation of her economic liberty and property rights. ECF No. 35 at 32. With regards to claims such as this one, the Supreme Court has enacted a three-part test for standing. First, the plaintiff must have suffered an injury in fact that is (1) concrete and particularized and (2) actual, imminent, and not

conjectural or hypothetical. Lujan v. Defenders of Wildlife, 504 U.S. 555, 560 (1992). Second, there must exist a causal connection between the injury and the complained conduct; the injury being fairly traceable to the challenged action of the defendant. Id. Third, it must be likely and not speculative that the injury will be redressed by a favorable decision by the court. Id. at 561.

Turning now to the circumstances of Ms. Vega's business, Hillside Cabin is still operating at twenty nights per month. Because Ms. Vega has operated Hillside Cabin for less than a year, she was unable to testify as to Hillside Cabin's "normal" capacity, only noting that for several months before the mandates, Hillside Cabin was completely booked. Likewise, she could not testify as to whether a decrease in her occupancy—if any—could be attributed to normal seasonality, initial novelty of her business, or other factors besides the mandates. More importantly, the mandates may not be having any impact at all on Hillside Cabin's occupancy because Ms. Vega did not state in her testimony whether she, as owner of Hillside Cabin, was even complying with EO75. Instead, when asked whether she was asking her guests for proof of vaccination she invoked her Fifth Amendment right to remain silent.[5] If Ms. Vega is not following the requirements of EO75, then EO75 cannot be inflicting any actual, concrete, and particularized harm to her business, and her alleged harm is therefore conjectural, speculative, and does not constitute injury in fact. Accordingly, Ms. Vega produced no evidence to demonstrate a substantial likelihood of standing to bring her economic liberty and property rights claim because she produced no evidence of either an actual, concrete, or particularized harm, nor did she show a causal connection between the mandate and her harm.

[5] Ms. Vega invoking her constitutional right under the Fifth Amendment is not being held against her. Nor is the court taking her silence as evidence that she is not complying with EO75's mandate. Instead, because a movant bears the burden of convincing the court that they merit the extraordinary remedy of a preliminary injunction, Ms. Vega has the burden of showing she was actually harmed by the mandate she challenges to establish standing. But by refusing to provide that evidence, Ms. Vega has failed in her burden to show a substantial likelihood of standing on her economic liberty and property rights claim.

2. Ripeness of Mr. Matos' Property Right Claim

Mr. Matos also argues that Regulation 138-A deprives him of his right to earn an honest living and threatens his property interest in his health certificate which he needs to continue working in his current position. ECF No. 35 at 31. However, Mr. Matos' harm to his property interest in his health certificate is not ripe for adjudication. For a claim to be ripe, two factors must be present: (1) the hardship to the parties of withholding court consideration, and (2) the fitness of the issues for judicial decision. Doe v. Bush, 323 F.3d 133, 138 (1st Cir. 2003). The hardship prong examines whether a plaintiff "is suffering any present injury from a future contemplated event." McInnis-Misenor v. Maine Med. Cntr., 319 F.3d 63, 70 (1st Cir. 2003). The fitness prong depends on "[t]he baseline question [of] whether allowing more time for development of events would significantly advance our ability to deal with the legal issues presented [or] aid us in their resolution." Bush, 323 F.3d at 138–39. Namely, an issue is not fit if many important questions remained unanswered and if the court will have to "pile one hypothesis on top of another" and assume that a certain future state of affairs will come to pass. See id. at 139.

Both prongs weigh against a finding that Mr. Matos' claimed property interest in his health certificate is ripe.[6] Withholding consideration of Mr. Matos' claim results in only minor hardship for Mr. Matos. Mr. Matos currently has a health certificate which he secured shortly after Regulation 138-A was promulgated in August 2021. Therefore, although he fears that his certificate may not be renewed in August 2022, he suffers no present injury regarding his employment because he currently holds a health certificate, and he continues to work.

[6] The court withholds judgment on the legal question of whether Mr. Matos has a property right in his health certificate. Even if Mr. Matos does have a property interest in his health certificate, his claim is not ripe.

Turning to the second prong, it is not even clear that Mr. Matos will ever lose his health certificate, and it is not a given that he will be denied a certificate in the future. For the court to conclude that Mr. Matos will be unable to renew his health certificate requires the assumption that the Puerto Rico government will still require COVID-19 vaccination as a requisite for obtaining a health certificate in August 2022. That assumption also requires the hypothesis that the COVID-19 pandemic and pandemic related restrictions will not improve before August 2022. Therefore, resolution of this issue is not fit because only more time will allow for the development of events that would significantly advance the court's ability to deal with the legal issues presented. In sum, Mr. Matos' fails to show a substantial likelihood of ripeness for his claimed for violation of his property right in his health certificate.[7]

b. Whether EO 75 and Regulation 138-A Violate Plaintiffs' Substantive Due Process Rights

Turning now to specifics of Plaintiffs' remaining claims, for the last century federal courts have developed and applied a detailed hierarchy of scrutiny when analyzing government action which allegedly led to violations to constitutional rights—rational basis scrutiny, intermediate scrutiny, and strict scrutiny being the most commonly invoked. County of Butler v. Wolf, 486 F. Supp. 3d 883 (W.D. Penn. 2020). Substantive due process challenges to vaccine mandates and COVID-19 measures have been analyzed by many courts under the scrutiny standard established by Jacobson v. Massachusetts, 197 U.S. 11 (1905). Many courts have interpreted Jacobson, a one-hundred-and-fifteen-year-old case that predates the tiers of scrutiny, to establish an extremely deferential standard toward government action during public health

[7] Ms. Llenza also charges that Regulation 138-A prevents her from obtaining a health certificate. ECF No. 35 at 29–30. In contrast to Mr. Matos, Ms. Llenza does not currently have a health certificate, and she therefore cannot claim to have a property interest in something she does not yet have. Accordingly, any asserted property interest by Ms. Llenza is also unripe.

emergencies, effectively requiring that government action during a pandemic always be analyzed under something akin to or lower than rational basis review. See e.g. Bimber's Delwood, Inc. v. James, 496 F. Supp. 3d 760, 774 (W.D.N.Y. Oct. 21, 2020) ("Indeed, the overwhelming majority of courts resolving constitutional challenges to COVID-19-related measures employ Jacobson."); League of Indep. Fitness Facilities & Trainers, Inc. v. Whitmer, 814 F. App'x 125 at 127–28 (6th Cir. 2020) ("[T]he police power retained by the states empowers state officials to address pandemics such as COVID-19 largely without interference from the courts"); see also County of Butler, 486 F. Supp. 3d at 896 ("Defendants argue that no matter which traditional level of scrutiny that the underlying constitutional violation would normally require, a more deferential standard is appropriate.").

Of course, because Jacobson predates the tiers of scrutiny, the words "rational basis" are never used in the opinion, and its holding has been subject to qualification and debate ever since. Accordingly, Plaintiffs argue that their claims should not be scrutinized under Jacobson, but rather that the court should apply "heightened constitutional scrutiny," particularly in regard to Plaintiffs' claimed violations of "personal . . . autonomy, bodily integrity, and medical choice." ECF No. 7 at 6–7. Defendants urge the court to apply the deferential Jacobson rational basis standard in this case, thereby preserving the executive orders and regulations under the Puerto Rico government's "broad police powers." ECF No. 20 at 4–5.

In Jacobson, the Supreme Court analyzed a claim by plaintiff Henning Jacobson when he challenged a state law which required him to submit to a smallpox vaccination during an ongoing smallpox pandemic, or else pay a $5 fine or establish that he was exempt. Jacobson, 197 U.S. at 12–14. The Supreme Court held that "the police power of a state must be held to embrace, at least, such reasonable regulations established directly by legislative enactments as will protect

the public health and the public safety." Jacobson, 197 U.S. at 25; see also Zucht v. King, 260 U.S. 174, 176 (1922) ("Jacobson v. Massachusetts . . . . had settled that it is within the police power of a state to provide for compulsory vaccination."). In finding that states may properly exercise the police power to address a pandemic, the Jacobson Court stated what is normally described as the "Jacobson standard," which approximates rational basis review: Courts may only "review," "adjudge" and "thereby give effect to the Constitution" with regard to government measures involving a public health emergency such as a pandemic if the measures "purporting to have been enacted to protect the public health, the public morals, or the public safety, [have] no real or substantial relation to those objects"[8] or if the government action "is beyond all question, a plain, palpable invasion of rights secured by the fundamental law" Jacobson, 197 U.S. at 31.

"Fundamental" rights or liberties are those protected by the Due Process Clause and include "most of the rights enumerated in the Bill of Rights . . . [I]n addition these liberties extend to certain personal choices central to individual dignity and autonomy, including intimate choices that define personal identity and beliefs." Obergefell v. Hodges, 576 U.S. 644, 663 (2015). Fundamental rights and liberties are "objectively deeply rooted in this nation's history and tradition" so that "neither liberty nor justice would exist if they were sacrificed." Washington v. Glucksberg, 521 U.S. 702, 720-21 (1997). These fundamental rights include "the right to marry," "to have children," "to direct the education and upbringing of one's children," "to marital privacy," "to use contraception," "to bodily integrity," and "to abortion . . . ." Id. at 721. In a substantive due process challenge, a plaintiff must provide a "careful description" of the asserted fundamental liberty interest. Id.

[8] Plaintiffs make no allegation, nor did they produce any evidence, that the challenged measures put in place by the Puerto Rico government have no real or substantial relation to public health.

Accordingly, Jacobson does not give free license to a government to trample on any constitutional right during a pandemic; instead, any such abrogation must examine the right at issue and whether a fundamental right is involved. In Roman Catholic Diocese v. Cuomo, the Supreme Court found that a COVID-19 regulation in New York that targeted houses of worship was subject to "strict scrutiny," because the regulations were an abrogation of the Free Exercise Clause of the First Amendment and were not religiously "neutral" and of "general applicability." Roman Catholic Diocese v. Cuomo, 141 S. Ct. 63, 67 (2020). Therefore, the New York restriction must have been narrowly tailored to serve a compelling state interest.

In his concurrence in Cuomo, Justice Neil Gorsuch went on to explain that the Supreme Court in Jacobson "essentially applied rational basis review to Henning Jacobson's challenge . . . ." because the right Jacobson asserted was different than the right the plaintiffs raised in Cuomo. Cuomo, 141 S. Ct. at 70 (Gorsuch, J., concurring). Unlike the plaintiffs in Cuomo, who invoked the Free Exercise right under the First Amendment, Jacobson had raised "a substantive due process right to bodily integrity that emanated from the Fourteenth Amendment . . ." Cuomo, 141 S. Ct. at 70 (Gorsuch, J., concurring) (internal citations omitted). Justice Gorsuch explained that

> "Rational basis review is the test this Court *normally* applies to Fourteenth Amendment challenges, so long as they do not involve suspect classifications based on race or some other ground, or a claim of fundamental right. Put differently, Jacobson didn't seek to depart from normal legal rules during a pandemic, and it supplies no precedent for doing so. Instead, Jacobson applied what would become the traditional legal test associated with the right at issue—exactly what the Court does today . . ."

Cuomo, 141 S. Ct. at 70 (Gorsuch, J., concurring) (emphasis in original). Therefore, the holding in Cuomo was consistent with Jacobson because the court applied the correct standard for the right in question: strict scrutiny was the correct standard because the restrictions in New

York were a plain, palpable invasion of the fundamental right secured by the First Amendment Free Exercise Clause, while the right Jacobson raised was not a fundamental right. See also Klassen v. Trustees of Indiana Univ., 2021 WL 3073926 at *21 (N.D. Ind. July 18, 2021) ("This view remains consistent with the right at stake in Jacobson: though a true "liberty" proved at stake—the right to refuse a vaccine during a smallpox epidemic . . . wasn't fundamental under the Constitution to require greater scrutiny than rational basis review").

Justice Gorsuch lamented that courts have mistakenly interpreted Jacobson for "towering authority that overshadows the Constitution during a pandemic[.]" Cuomo, 141 S. Ct. at 71;[9] see also Klassen, 2021 WL 3073926 at *21 ("Jacobson didn't hold that the government's authority in a pandemic balloons for it do whatever it wants in the name of public safety.") Indeed, the Jacobson Court itself explained that public health measures during a pandemic may not always be constitutional. The Jacobson Court cautioned,

> it might be that an acknowledged power of a local community to protect itself against an epidemic threatening the safety of all might be exercised in particular circumstances and in reference to particular persons in such an arbitrary, unreasonable manner, or might go so far beyond what was reasonably required for the safety of the public, as to authorize or compel the courts to interfere for the protection of such persons.

Jacobson, 197 U.S. at 28. The Court went on and explained,

> Before closing this opinion we deem it appropriate, in order to prevent misapprehension as to our views, to observe—perhaps to repeat a thought already sufficiently expressed, namely— that the police power of a state, whether exercised directly by the legislature, or by a local body acting under its authority, may be exerted in such circumstances, or by regulations so arbitrary and oppressive in particular cases, as to justify the interference of the courts to prevent wrong and oppression."

[9] Justice Gorsuch went on to write "In the end, I can only surmise that much of the answer lies in a particular judicial impulse to stay out of the way in times of crisis. But if that impulse may be understandable or even admirable in other circumstances, we may not shelter in place when the Constitution is under attack." Roman Catholic Diocese, 141 S. Ct. at 71.

Jacobson, 197 U.S. at 38.

In sum, Jacobson does not function as a rubber stamp for government action during a pandemic; rather, the standard of scrutiny depends on the right being asserted. See Cuomo, 141 S. Ct. at 68 ("But even in a pandemic, the Constitution cannot be put away and forgotten. The restrictions at issue here, by effectively barring many from attending religious services, strike at the very heart of the First Amendment's guarantee of religious liberty."). The First Circuit likewise recognized that Jacobson does not remove every right from the normal standards of scrutiny during a pandemic. See Does 1-6 v. Mills, 16 F.4th 20, 29 (1st Cir. 2021) (recognizing in dicta that during the context of the COVID-19 pandemic a law that is not religiously neutral or of general applicability will be subject to strict scrutiny).

Plaintiffs' claims should be closely analyzed based on the right being asserted, whether the claim rises to a fundamental right or whether the asserted liberty interest only merits rational basis review. To show a likelihood of succeeding on the merits, plaintiffs must show that the government action cannot survive the requisite scrutiny associated with the asserted right or must convince the court that the government acted in an arbitrary, oppressive, unreasonable manner, going far beyond what was reasonably required for the safety of the public. Jacobson, 197 U.S. at 28, 31.

1. Personal Autonomy, Bodily Integrity, and Medical Decision-making Claims

Turning now to the rights at issue, Plaintiffs Ms. Vega, Ms. Llenza, and Mr. Matos claim that the requirement for vaccine or COVID-19 test checks in public places under EO75 violates their constitutional rights to personal autonomy, bodily integrity, and medical choice under the Fourteenth Amendment because they must either be vaccinated or submit to a COVID-19 test

whenever they wish to "attend restaurants, bars, get a haircut, or stay in a hotel or Airbnb, among other activities." ECF No. 35 at 32.

The Supreme Court has unquestionably recognized a liberty interest in the right to "bodily integrity," and that the Due Process Clause protects the traditional right to refuse unwanted lifesaving medical treatment. Glucksberg, 521 U.S. at 720 citing (Cruzan v. Dir., Missouri Dep't of Health, 497 U.S. 261, 278–79 (1990)). However, since Jacobson, the Supreme Court has not identified the right to refuse vaccination as implicating a fundamental right meriting any more scrutiny than rational basis. See Cuomo, 141 S. Ct. at 70.

The Supreme Court is "reluctant to expand the concept of substantive due process" to specific liberties in part because "[b]y extending constitutional protection to an asserted right or liberty interest, we, to a great extent, place the matter outside the arena of public debate and legislative action. We must therefore exercise the utmost care whenever we are asked to break new ground in this field . . . ." Glucksberg, 521 U.S. at 721 (internal quotations omitted).

The right to refuse a vaccine, although implicating an interest in bodily autonomy protected by the Constitution, does not constitute a fundamental right that would require greater scrutiny than the low standard set by Jacobson. Klassen, 2021 WL 3073926 at *21. Therefore, although Plaintiffs have a right to bodily integrity, consistent with the precepts of Glucksberg, Jacobson, and Cuomo, the right to refuse vaccination does not implicate a fundamental right of bodily integrity which merits higher scrutiny than rational basis. Klassen v. Trustees of Indiana University, 7 F.4th 592, 593 (7th Cir. 2021) ("[T]here can't be a constitutional problem with vaccination against SARS-CoV-2").

If vaccination against COVID-19 does not violate a fundamental right to bodily integrity and medical choice, then the alternatives under EO75 also do not rise to the level of fundamental

violations. See id. ("These plaintiffs just need to wear masks and be tested, requirements that are not constitutionally problematic."). If vaccination, as the permanent injection of a foreign substance into the patient's body, does not implicate a right to bodily integrity and medical choice, then certainly the temporary insertion of a swab into a patient's nose does not either. In fact, Plaintiffs Llenza and Matos do not object to COVID-19 tests as strongly as they do to vaccination, because although both Plaintiffs refuse to be vaccinated, they both testified that they regularly submit to free COVID-19 tests. Only Plaintiff Ms. Vega objects to the tests on uncertain religious grounds that will be discussed below. In sum, to withstand Plaintiffs' challenges based on bodily autonomy and medical choice, the vaccine check and COVID-19 test result checks under EO75 must merely survive rational basis review.

2. Privacy Rights Claims

Second, Plaintiffs Ms. Vega, Ms. Llenza, and Mr. Matos further claim that being required to show proof of vaccination or a COVID-19 test violates their constitutionally protected medical privacy rights. ECF No. 35 at 32. Ms. Vega additionally claims, alongside Tropical Chill, that EO75 unconstitutionally requires those Plaintiffs to violate the constitutional privacy rights of their customers when they ask for proof of vaccination or a negative COVID-19 test. ECF No. 35 at 32.

"The privacy interest in medical information is neither fundamental nor absolute." United States v. Kravetz, 706 F.3d 47, 64 (1st Cir. 2013) (citing Whalen v. Roe, 429 U.S. 589, 878 (1977)). In fact, under normal circumstances, requiring disclosures of medical information "to representatives of the State having responsibility for the health of the community, does not automatically amount to an impermissible invasion of privacy." Whalen, 429 U.S. at 878. Because medical privacy rights are not "fundamental," government action infringing on those

rights during a pandemic should fall squarely within the rational basis review normally required and demanded by Jacobson. Klassen, 2021 WL 3073926 at *16 ("If the government infringes on a fundamental right, the court often applies strict scrutiny . . . Whereas infringements on other rights or liberties, though still constitutionally scrutinized, must meet what courts call rational basis review.") (citing Glucksberg, 521 U.S. at 722; Sweeney v. Pence, 767 F.3d 664, 668 (7th Cir. 2014)).

3. Economic Liberty and Property Rights Claims

Finally, Plaintiffs bring economic liberty and property rights claims. In particular, Plaintiffs Tropical Chill and Ms. Vega argue that the requirements under EO75 that they ask for proof of vaccination or a negative COVID-19 test—or for Tropical Chill, to operate at 50 percent capacity—infringes upon their right to earn a living and use their property as they see fit. ECF No. 35 at 32. Ms. Llenza also argues that the vaccination requirements under EO75 and Regulation 138-A infringe upon her ability to obtain employment in the fields for which she has been trained, in violation of her economic liberty rights. ECF No. 35 at 29–30.

It has long been held that "the right to work for a living in the common occupations of the community is of the very essence of the personal freedom and opportunity that it was the purpose of the [Fourteenth] Amendment to secure." Truax v. Raich, 239 U.S. 33, 41 (1915). However, economic liberty and property rights claims do not rise to the level of fundamental rights warranting heightened scrutiny because the Supreme court has "held for many years (logically or not) that the 'liberties' protected by substantive due process do not include economic liberties." Stop the Beach Renourishment, Inc. v. Florida Dept. of Environmental Protection, 560 U.S. 702, 721 (2010). This is not to say that these interests are not constitutionally protected; however, judicial review only scrutinizes infringements of these rights under rational basis review. See

United States v. Carolene Products, 304 U.S. 144, 153 (1938) (Applying rational basis scrutiny to "regulatory legislation affecting ordinary commercial transactions.")

Additionally, "[t]he right to hold private employment and to pursue one's chosen profession free from unreasonable government interference is encapsulated in the liberty concept of the Due Process Clause. Mead v. Independence Ass'n, 684 F.3d 226, 232 (1st Cir. 2012). However, the First Circuit has noted that this right is usually "implicated only by government interference that is direct and unambiguous, as when a city official demands that a restaurant fire its bartender, . . . or a state agency explicitly threatens to prosecute a private company's clients if they continue to contract with the company." Id. Substantive due process challenges based on the right to work in a chosen profession are therefore properly analyzed under the rational basis standard that applies to economic liberties. County of Butler, 486 F. Supp. 3d at 920–21 ("[C]ourts generally treat government action purportedly violating the right to pursue an occupation in the same light as economic legislation and use the general standard of review applied to substantive due process claims . . . Substantive due process challenges to a legislative act are reviewed under the rational basis test."). Therefore, EO75 and Regulation 138-A, as challenged by Plaintiffs economic liberty and property rights claims, should also be analyzed under rational basis scrutiny.

4. Whether the Mandates Withstand the Requisite Scrutiny under Jacobson

Insofar as EO75 and Regulation 138-A do infringe on Plaintiffs' substantive due process rights, to demonstrate a substantial likelihood of success the Plaintiffs bear the burden of showing that the mandates are not rationally related to a legitimate government interest or that the government acted in an arbitrary, oppressive, unreasonable manner, going far beyond what was reasonably required for the safety of the public. Jacobson, 197 U.S. at 28, 31; Medeiros v.

Vincent, 431 F.3d 25, 29 (1st Cir. 2005) ("Legislation or regulation which neither employs a suspect classification nor impairs fundamental rights, will survive constitutional scrutiny, provided the remedy is "rationally related" to a legitimate governmental purpose.").

The Supreme Court has clearly stated that "[s]temming the spread of COVID-19 is unquestionably a compelling state interest . . . ." Cuomo, 141 S. Ct. at 67. Likewise, the First Circuit has declared: "Few interests are more compelling than protecting public health against a deadly virus." Does 1-6 v. Mills, 16 F.4th 20, 32 (1st Cir. 2021). That the government has a "compelling interest" constitutes a high enough interest for strict scrutiny, and therefore, certainly high enough for rational basis. The only remaining question is whether EO75 and Regulation 138-A are "rationally related" to that compelling government interest or whether the government acted in an arbitrary, oppressive, unreasonable manner, going far beyond what was reasonably required.

In this analysis, it is of paramount importance that courts refrain from policymaking, principally because the Constitution "entrusts the safety and the health of the people to the politically accountable officials of the States." S. Bay United Pentecostal Church, 140 S. Ct. 1613 (2020) (Roberts, C. J., concurring). Furthermore, courts "should respect the judgment of those with special expertise and responsibility in this area." Cuomo, 141 S. Ct. at 68. Therefore, "[i]t is no part of the function of a court or a jury to determine which one of two modes was likely to be the most effective for the protection of the public against disease." Jacobson, 197 U.S. at 30. Courts must not therefore subject public health mandates "to second-guessing by an 'unelected federal judiciary,' which lacks the background, competence, and expertise to assess public health and is not accountable to the people." S. Bay United Pentecostal Church, 140 S. Ct. at 1613–14 (Roberts, C. J., concurring).

Based on the arguments of the parties, relevant sources of authority, documentary evidence, and testimony of the witnesses at the preliminary injunction hearing, both Regulation 138-A and EO75 are rationally related to a legitimate government interest and were not enacted with a degree of arbitrariness, oppression, and unreasonableness that far exceeds what was required for the public's safety.

i. Executive Order 75 Vaccination Check Requirement for Businesses

Plaintiffs challenge EO75 and its mandate that private businesses require COVID-19 vaccination or weekly tests for individuals working in the private sector, such as Mr. Matos who works for a supermarket, and Ms. Llenza, who is seeking employment in sectors which would require weekly testing. ECF No. 35 at 29–30, 31. Plaintiffs argue that getting the tests on a weekly basis is prohibitively costly and extremely burdensome. ECF No. 7 at 11–12. However, the evidence presented at the hearing is not as definitive. Both Mr. Matos and Ms. Llenza have almost always been able to secure free testing requiring only a few hours of time-investment each week.

Mr. Matos reached a verbal agreement with his employer whereby he will get Thursdays off to complete his COVID-19 testing rather than becoming vaccinated. Mr. Matos travels to Cabo Rojo to be tested, which requires him to drive approximately 20 minutes both ways. Once in Cabo Rojo, Mr. Matos will get tested either at the government "tracing center" or he will coordinate an electronic drive-through appointment at the local Walgreens Pharmacy. Neither option is particularly convenient for Mr. Matos. When Mr. Matos goes to be tested at the tracing center, he usually must wait in line for as long as one to two hours to be tested. Plaintiffs introduced photos that Mr. Matos took on various dates demonstrating the length of the line at the Cabo Rojo tracing center, and the outdoor line can be seen to extend for what appears to be

several storefronts. Exhibits 32–38. Mr. Matos also explained that the Cabo Rojo tracing center sometimes runs out of tests before all can be attended.

Mr. Matos' alternative to the testing center is the Cabo Rojo Walgreens. Mr. Matos explained that Walgreens requires him to coordinate a test appointment online which is then completed through the drive through window. Upon arrival, he sometimes must wait for 20 minutes to an hour to be served. While this process is easier than that offered at the tracing center, Mr. Matos explained that because of a quirk in Walgreens' online system, he cannot schedule consecutive tests on Thursday—his day off—because the system only allows for one free test per seven days. If one week Mr. Matos is tested at Walgreens Pharmacy on a Thursday, he can only schedule the next test for the following Friday and so is unable to schedule a free test on his next Thursday off. By getting tested in Cabo Rojo, either at the tracing center or at Walgreens, Mr. Matos testified that it takes between 2 and 2.5 hours to drive, park, wait in line, get tested, and return home. When asked, Mr. Matos characterized the testing regime neither as a mere inconvenience nor as a burden, but as "torture." Hearing, Dec. 7, at 5:19 PM.

Mr. Matos explained that he gets tested either at the Cabo Rojo tracing center or at Walgreens for reasons of cost. If he is tested only once a week at either the tracing center or at Walgreens, the tests are free for Mr. Matos. However, Mr. Matos also explained that if he cannot secure a test during the required time, he must get a test at a private lab which costs him $45. Mr. Matos's job pays him $8 an hour, and he does not have medical insurance. Therefore, Mr. Matos said he has only paid for a private test once after an outbreak occurred at work. Regularly using a private lab for weekly tests would be too onerous for Mr. Matos' limited income. Nevertheless, Mr. Matos has declined to request the health insurance plan offered by the government of Puerto Rico—Plan Vital.

Similarly, Ms. Llenza has so far been able to obtain free testing when required rather than getting vaccinated. Ms. Llenza reported that she tries to avoid the test taken using a nose swab because her eye had bled once after a rough nose test. She therefore goes to a lab in Carolina, Puerto Rico, that collects saliva tests. To arrive at the lab, Ms. Llenza must leave early in the morning, drive 25 to 30 minutes, and then drive home for an hour in rush-hour traffic. Ms. Llenza has only been tested in the lab in Carolina and testing there is free for her through her health insurance plan. Ms. Llenza testified that she became aware that free tests were also available at Walgreens through word of mouth, and that the government was offering free tests after she called a number associated with a Facebook ad posted by the Department of Health. Ms. Llenza also described how she once attempted to get a test done at one of the free government testing sites but that she was unable to have the test done because she arrived too late. To her knowledge, the government test sites usually operate one day a week from 8:00 AM to 1:00 PM, function on a first-come-first-served basis, and that they sometimes run out of tests before everyone in line can be tested. She has not used the free tests available at the Walgreens Pharmacy chain because she has found the process for setting up a drive-through appointment too tedious.

As discussed above, the Puerto Rico government has a rational basis in requiring testing for people who work in industries like Mr. Matos and for the fields in which Ms. Llenza has training. Plaintiffs certainly describe a process that is inconvenient as a weekly undertaking. However, contrary to Plaintiffs' assertions, in all but one instance, both Mr. Matos and Ms. Llenza have been able to be tested for free. They have also been able to travel relatively short distances to local testing centers with a time investment of roughly three hours. Such processes are not oppressive, and certainly not torturous as Mr. Matos testified.

ii. Executive Order 75 Vaccination Check Requirements

Plaintiffs argue that placing restrictions on unvaccinated persons' ability to frequent restaurants and other places covered by EO75 cannot be justified because "vaccinated people can get and spread infection []just like vaccinated people." ECF No. 7 at 17. They argue that the restrictions affecting their liberty interests were arbitrary, oppressive, and unreasonable, going far beyond what is reasonably required for the safety of the public because "the Puerto Rico[ ] health care system has never been in real jeopardy of being overwhelmed even during the worst part of the pandemic" and "as more people get vaccinated, the share of cases, hospitalizations, and deaths represented by unvaccinated people will tend to fall . . ." ECF No. 7 at 19. Therefore, Plaintiffs conclude that requiring Plaintiffs like Tropical Chill and Ms. Vega to check vaccination and COVID-19 test status is "irrational and arbitrary." ECF No. 35 at 12.

Plaintiffs contend that unvaccinated and vaccinated persons transmit COVID-19 equally because the viral load of vaccinated and unvaccinated people infected with COVID-19 is similar and "viral load is the most significant factor" in the ability for a person to infect. ECF No. 7 at 15. During the evidentiary hearing, Plaintiffs introduced the testimony of Dr. María E. Carrascal Muñoz, an expert in immunology and infectious diseases, who stated that in her clinical experience both vaccinated and unvaccinated persons who contract the COVID-19 virus will transmit it equally if they are symptomatic. Hearing Dec. 8, at 11:54 PM; ECF No. 80 at 1.

However, Dr. Carrascal's testimony conflicted in part with that of Plaintiffs' expert in epidemiology, Dr. Andrew Bostom. ECF No. 76 at 1. Dr. Bostom testified that persons who have been vaccinated may have a short period of time at peak vaccination where their ability to spread the COVID-19 virus may be less than an unvaccinated person, and that in randomized control trials vaccinated persons also got fewer infections. Both Doctors Carrascal and Bostom referred

to a study comparing how vaccinated and unvaccinated persons transmitted the COVID-19 virus in prison. Exhibit 52. Nevertheless, the utility of the study for society as a whole is questionable because, as Dr. Carrascal explained, it examined "a contained place that is closed and the people are very together, like in a prison." Hearing Dec. 8, at 11:54 PM.

During their presentation of the evidence, Dr. Iris Cardona Gerena testified as an expert in public health, immunizations, infectious diseases, vaccine preventable diseases, and pediatry. ECF No. 82 at 1. Dr. Cardona confirmed that for the first four days of infection by the COVID-19 virus, persons who are unvaccinated and vaccinated both have the same viral load. However, she clarified that after the first four days, the viral load decreases faster in vaccinated individuals, which means that the unvaccinated can transmit the virus for a longer period of time. On this same point, Defendants also introduced the testimony of Dr. Melissa Marzán Rodríguez who was admitted as an expert in epidemiology and public health. ECF No. 84 at 1. Dr. Marzán confirmed on cross examination that viral load is the most important factor in transmitting the COVID-19 virus, but that the viral load in vaccinated people decreases more quickly than those who are not vaccinated.

In terms of risk of contracting COVID-19, Defendants introduced data compiled by Dr. Marzán which showed that unvaccinated persons were 2.49 times more likely to contract COVID-19 than an unvaccinated person in August 2021—at the height of the Delta variant spike. Exhibit D, at 1. The same data showed that an unvaccinated person was 5.72 times more likely to be hospitalized from COVID-19 in August 2021, and 7.02 times more likely to die from COVID-19. Exhibit D, at 2–3. Finally, Defendants presented the testimony of Dr. Rafael Irizarry, admitted as an expert in biostatistics, who testified that the risk for unvaccinated people to contract COVID-19 is more than 10 times higher compared to a person who was vaccinated.

Dr. Irizarry concluded that unvaccinated people are much more likely to be infected and hospitalized because of COVID-19. The testifying experts agreed that a vaccinated person's protection wanes six months after being vaccinated. However, Dr. Cardona clarified that although vaccine protection from infection wanes after six months, the protection against hospitalization and death remained high.

The evidence further showed that restrictions on unvaccinated persons' access to the locations covered under EO75 are not arbitrary. Dr. Cardona testified that in closed environments, particularly in spaces that are small, the COVID-19 virus can be extremely contagious. Dr. Cardona, who also serves as an adviser to the Secretary of Health and as a member of the Scientific Coalition appointed by the Governor of Puerto Rico, reported that there is a greater risk of transmission in enclosed spaces where people are eating and conversing without masks. Hearing, Dec. 9, at 1:35 PM. She went on to say that enclosed spaces could include Airbnb's, schools, movie theaters, restaurants, and gyms. Hearing, Dec. 9, at 1:36–1:38 PM. Defendants' biostatistician expert witness, Dr. Irizarry also explained that according to his statistical analysis, "the one place that comes out very clearly" in the data as a place where he sees more infections are "restaurants . . . . This is a place that is indoors, and you take your mask off." Hearing, Dec. 14, at 10:27 AM. He continued, "[T]he second highest that we saw were gyms, which are similar, you are indoors and you are breathing heavily." Hearing, Dec. 14, at 10:27–10:28 AM.

Both parties also introduced evidence regarding the positive correlation between the number of COVID-19 cases in Puerto Rico and hospitalizations, ICU referrals, and deaths—collectively labeled "adverse health outcomes." Dr. Joel Hay also testified as an expert witness for the Plaintiffs, admitted as an expert in public health economics. ECF No. 76 at 1. Dr. Hay

raised a principle called "Farr's law." Dr. Hay asserted that Farr's law is a "theme with most infectious agents. Over time, the virus, or the infectious agent . . . adapts to the host [and] becomes less lethal over time." Hearing, Dec. 6, at 11:06 AM. Therefore "through the natural process of, you might even call it evolution, the virus changes and becomes less lethal and more infectious." Hearing, Dec. 6, at 11:08 AM. Because of Farr's law and increased numbers of vaccinated people, Dr. Hay predicts that the number of COVID-19 cases will eventually cease to have an impact on the number of hospitalizations, ICU referrals, and deaths.

Defendants' experts, however, testified that if Farr's law applies to COVID-19, it has yet to eliminate the correlation between positive cases and adverse health outcomes. Dr. Cardona agreed that there is evidence that viruses do decline in deadliness over time, but that mutations in viruses do not guarantee this trajectory. Viruses, Dr. Cardona explained, can mutate to become both more infectious and more severe, and she referred to the example of the H1N1 swine flu in 2009. Hearing, Dec. 9, at 11:07 AM. Dr. Marzán, Defendant's expert epidemiologist, also acknowledged that it can be the behavior of certain infectious agents to become less lethal and more transmissible over time. Nevertheless, Dr. Marzán and Dr. Irizarry highlighted that the Delta wave, which was the second-to-last wave experienced in Puerto Rico before December 2021, was substantially more deadly than the wave the preceded it.[10] Dr. Irizarry therefore expressed doubt that Farr's law has manifested itself in Puerto Rico's COVID-19 outbreak.

Even assuming Farr's law applies to the COVID-19 virus—an opinion upon which the experts were split—the positive effect of the law has yet to be seen in Puerto Rico. Defendants' biostatistician, Dr. Irizarry, testified regarding two graphs he compiled using Puerto Rico's public data on the number of hospitalizations and deaths. Dr. Irizarry reported, and both graphs

[10] Plaintiff's public health economics expert, Dr. Hay, also acknowledged that the Delta wave was more deadly than the wave that preceded it.

clearly demonstrate, that for the last two COVID-19 outbreak "waves" in Puerto Rico, there continues to be a remarkably strong correlation between the number of cases and the number of hospitalizations and deaths. Exhibits F, G. Dr. Irizarry showed that a rise in positive COVID-19 cases "predicts hospitalizations that are going to happen in one or two weeks in the future." Hearing, Dec. 14, at 9:26 AM; Exhibit F. Likewise, Dr. Irizarry explained that an increase in the positivity rate in Puerto Rico precedes an increase in deaths by "between two and three weeks." Hearing, Dec. 14, at 9:29 AM; Exhibit G. It was Dr. Irizarry's opinion that using this predictive information, when case numbers begin to grow the Puerto Rico government has been able to implement restrictions which have limited the growth in cases and therefore limited the total number of hospitalizations and deaths. Dr. Cardona agreed with Dr. Irizarry. She conveyed that by interrupting transmission of the virus, public health officials were able to avoid greater numbers of hospitalizations and death that pose a threat of overwhelming the Puerto Rico health system.

Plaintiffs disagree that Puerto Rico's health system has ever been at risk, and Dr. Hay testified to that effect alluding to a graph that illustrates the percentage of hospital and ICU beds in Puerto Rico occupied by COVID-19 patients, the percentage of hospital and ICU beds occupied by other patients, and the percentage of available hospital and ICU beds. Exhibit 11. He asserted that since the beginning of the Pandemic in August 2020, whenever there was a "spike" in COVID-19 hospitalizations there was a "one-for-one decline in hospitalization for other reasons" so that "hospitals have stayed at 60 percent occupancy" for the entire pandemic period. Hearing, Dec. 6, at 11:17 AM; Exhibit 11. Based on that data, Dr. Hay concluded that hospitals in Puerto Rico have never been in danger of being overwhelmed.

Defendants' expert Dr. Cardona, on the other hand, opined that there was the "possibility" that the health system in Puerto Rico would have collapsed without greater restrictions. She testified that "the risk of putting the system in danger is established when the occupancy [in hospitals] is greater than 70 percent." Hearing, Dec. 9, at 2:48 PM. This level of occupancy is only 10 percent points higher than the occupancy Puerto Rico hospitals have maintained during the pandemic, according to the graph alluded to by Dr. Hay. Exhibit 11. Dr. Cardona estimated that there was a possibility that 600 to 700 COVID-19 hospitalizations in Puerto Rico would not allow for the treatment of other patients requiring immediate attention.

Dr. Marzán, Defendants' expert epidemiologist who also serves as the Chief Epidemiologist for the Puerto Rico Department of Health, also testified as to the risk of overwhelming hospitals in Puerto Rico. Dr. Marzán asserted that it is not useful to talk about an island-wide collapse of the health system, because the threat may be highest to hospitals on a local or regional level, especially small hospitals. She noted that in August 2021, during the Delta variant outbreak, there were individual hospitals that were unable to admit any more COVID-19 patients. Dr. Marzán also reported that recent health emergencies in Puerto Rico, such as that which followed Hurricane María, compromised the Puerto Rico health system, which led the Puerto Rico government to implement the greatest number of measures as possible to avoid a similar crisis.

In sum, the evidence shows that vaccination does appear to provide some heightened level of protection, albeit temporarily, against spreading the COVID-19 virus, because a vaccinated person's viral load decreases more rapidly after they contract the virus, making them less likely to spread the virus. A vaccinated person is also less likely to contract COVID-19 and be hospitalized or die due to the infection. Because unvaccinated persons pose a heightened risk

of infection, the government of Puerto Rico has an interest in preventing unvaccinated individuals from entering high-risk locations without proof of a negative test or a COVID-19 infection within the last three months. It was also not irrational, arbitrary, and beyond what is reasonably required for the government of Puerto Rico to center these restrictions on places like restaurants, bars, movie theaters, and stadiums where people may congregate together and remove their masks to eat and drink. As testified by several experts, these places pose a particular risk for infection.

The government of Puerto Rico has a compelling interest in preventing increasing spread of the COVID-19 virus because, as shown in the evidentiary hearing, there remains a strong correlation between number of infections and adverse health outcomes. In other words, there is not yet any evidence that a significant increase in cases will not produce a significant number of hospitalizations, ICU referrals, and deaths which pose a risk to overwhelming hospitals. The Puerto Rico government has a particular interest in ensuring that the health system does not become overwhelmed on the island, particularly because, as Dr. Cardona testified, Puerto Rico's status as an island makes it much more difficult to transport patients who require hospitalization to other states or territories. Thankfully, it appears that the Puerto Rico health system has never reached the point of collapse during this pandemic, but the evidence adduced during the hearing shows that outcome continues to be a possibility. Indeed, as the evidence demonstrated, collectively Puerto Rico's hospitals may have only had a 10 percentage point margin of occupancy before a risk of collapse might have become reality. Accordingly, while there continues to be a strong correlation between more COVID-19 cases and adverse health outcomes, the government of Puerto Rico has an interest in regulating access to places where spread may be especially prevalent to prevent overwhelming the health system.

Many of Plaintiffs' complaints center on a disagreement on policy with the government of Puerto Rico. Plaintiffs suggest, as a matter of policy, that ice cream shops like Tropical Chill, where patrons only spend a few minutes, and Ms. Vega, who makes no physical contact with her guests, should be subject to some sort of exemption. However, these cases illustrate the difficulty in crafting enforceable public policy to cover a broad variety of businesses. Plaintiffs also argue that requiring workers to test on a weekly basis, while requiring visitors to covered businesses to test 72 hours in advance shows that EO75 is arbitrary and capricious. ECF No. 35 at 14.[11] Plaintiffs also disagree that the challenged measures have effectively prevented overwhelming the Island's hospitals—and it is admittedly difficult to tell. [12]

However, the Puerto Rico government must draw lines with its policies, and a policy may still be rationally related to a legitimate government interest even if the policy is "not perfectly tailored to that end." Box v. Planned Parenthood of Indiana and Kentucky, Inc., 139 S. Ct. 1780, 1782 (2019) ("[T]he state need not have drawn 'the perfect line,' as long as 'the line actually drawn [is] a rational' one.") (internal citations omitted). As explained above, the specific policies being questioned in this case are rationally related to the compelling government interest of stopping the spread of the pandemic, and Plaintiffs have failed to show that they are arbitrary and far beyond what is reasonably required. The government of Puerto Rico has drawn rational lines, even if not perfect lines. Therefore, it is not proper for a court to second-guess the public health

[11] An argument could be made, however, that it is reasonable to impose more frequent testing on optional activities than for those, like employment, upon which people depend for their livelihood. Furthermore, unlike places covered by EO75 (e.g. bars, restaurants, etc.), which pose a higher risk because they are often enclosed spaces in which people generally remove their masks, not all workplace environments require removal of a mask in an enclosed space.

[12] The tension between the Plaintiffs and Defendants' views is best summarized as follows: From Plaintiffs' perspective, because Puerto Rico's health care system has never been overwhelmed, it is arbitrary, irrational, oppressive, and unreasonably burdensome for the Government of Puerto Rico to impose the restrictions and mandates found in EO75 and Regulation 138-A. Defendants, on the other hand, contend that it is precisely because of these tight restrictions and strict mandates that Puerto Rico's health system has not been overwhelmed.

experts on measures—even if more exceptions or fewer restrictions could be a more attractive policy option. In sum, restrictions on unvaccinated persons' activities in places covered by EO75 are rationally related to Puerto Rico's compelling health goals, and the mandates—though not necessarily without flaws and totally justifiable should circumstances change—are not arbitrary, oppressive, or unreasonable to a degree that fails to pass constitutional muster.

iii. Regulation 138-A

Plaintiff Llenza challenges Regulation 138-A because to work as a professional food manager, she is required to obtain a health certificate which she cannot do because she is unvaccinated. ECF No. 35 at 29–30. Ms. Llenza claims that she should be able to receive a health certificate because she had previously contracted COVID-19 and so has natural immunity. In support of that claim, Plaintiffs continually sought to introduce evidence that natural immunity is as robust and long lasting as vaccine induced immunity or better. For example, a recent study from Israel concluded that "natural immunity confers longer lasting and stronger protection against infection, symptomatic disease and hospitalization caused by the Delta variant of SARS-CoV-2 . . . ." Exhibit 46 at 2. The experts presented by both Plaintiffs and Defendants agreed that vaccine immunity lasts for only about six months, which is why booster shots have been developed. However, one of Plaintiffs' expert witnesses, Dr. Carrascal, went so far as to suggest that immunity for COVID-19, like immunity for measles and chickenpox, lasts for the entirety of a person's life after they were infected. Such an assertion lacks credibility in light of the other evidence, and Dr. Carrascal herself admitted that antibodies for COVID-19 decrease in a person over time. One of Defendants' expert witnesses, Dr. Cardona, testified that it is not yet clear how long natural immunity from COVID-19 infection actually lasts, and that it could last as long as 13 months. However, Doctors Cardona, Marzán, and Irizarry all reported that there are

documented cases of persons who contracted COVID-19 becoming reinfected with COVID-19 after 90 days.

In addition to the medical testimony, Plaintiffs also presented the testimony of lay witness Ofelia Otero Santiago ("Ms. Otero") who had a truly horrific experience with the first dose of the COVID-19 vaccination. Ms. Otero contracted COVID-19 in November 2020. After recovering from her COVID-19 infection, Ms. Otero received the first dose of the Pfizer vaccine in August 2021. In the days following her vaccination, Ms. Otero's left side of her body became swollen, she suffered pain from her head to her feet, and was unable to sleep for days. Her left side of her body also became paralyzed, and she began bleeding from burst capillaries in her left eye. Eventually, Ms. Otero passed out from her symptoms and had to be hospitalized. After this ordeal, Ms. Otero was counseled by several doctors who told her that her reaction was a result of excessive immunization. Dr. Cardona acknowledged that past scientific literature does speak of a hyperimmune response in persons who received too many doses of a tetanus vaccine in a short period of time.

Understandably, Ms. Otero does not want to get a second dose of a COVID-19 vaccine because of her extreme reaction to the first dose. Ms. Otero testified that before her adverse reaction to the vaccination, she was planning on opening a sportswear business. Ms. Otero believes that due to her situation she would not be able to obtain a health certificate under Regulation 138-A because she is not yet "fully vaccinated" after having only received one dose of the vaccine. Ms. Otero, however, has not attempted the process of obtaining a health certificate.

Perhaps the implication of this evidence is that Regulation 138-A should provide some sort of exemption to obtain a health certificate for someone who has contracted and recovered

from COVID-19 and who has natural immunity. However, the only Plaintiff who fits this description is Ms. Llenza. Ms. Llenza contends that because she has already contracted COVID-19, she has immunity from the virus, no longer needs to be vaccinated, and should be able to obtain a health certificate. In support of this, Plaintiffs introduced two lab tests from June 25 and September 17, 2021 which show that Ms. Llenza still had antibodies for the SARS-CoV-2 virus. Exhibit 43, 44.

Nevertheless, the Puerto Rico government, firstly, has a rational basis in requiring a health certificate for people who work in industries like that of Mr. Matos and occupations for which Ms. Llenza has received training. Mr. Matos works in food distribution, while Ms. Llenza is seeking employment in disaster recovery, food preparation, or medical billing. In all these industries, there is a rational and reasonable need for persons to have a health certificate to avoid spreading illness to others.[13]

Secondly, it does appear likely that natural immunity provides protection against COVID-19. However, as discussed above, the Plaintiffs failed to clarify with any certainty how long natural immunity may last, while Defendants' produced evidence that it may only last as long as 90 days. Based on this evidence, it is not arbitrary, for example, that EO75 specifies that a person with natural immunity may also enter the establishments covered by EO75 within three months of infection. However, Regulation 138-A does not provide an exemption whatsoever for persons with natural immunity unless an applicant for a health certificate can provide evidence that "the patient has a compromised immune system or there is a medical contraindication that prevents inoculation. This must be certified by a doctor authorized to practice in Puerto Rico or

[13] It is puzzling and difficult to see the rational basis as to why someone like Ophelia Otero would need to have a health certificate to open a clothing business, but because Ms. Otero is not a Plaintiff, the court cannot rule on that issue. Furthermore, even if Ms. Otero were a plaintiff, at the preliminary injunction hearing she testified that she is no longer currently seeking to open the clothing business and thus, has not sought a health certificate.

by the doctor who issues the health certificate." ECF No. 35-1 at 3. This type of exemption, Dr. Cardona testified, would presumably apply to a person like Ms. Otero, as long as a doctor provided a sufficient basis for why the person should not be vaccinated. Ms. Llenza, however, has not produced any evidence showing that there is a medical reason for her not to be vaccinated, and she has provided no evidence to believe she is at risk of having an immune reaction like Ms. Otero. Once again, this is a situation where the government of Puerto Rico has inevitably had to draw lines on what persons qualify for an exemption to vaccination for a health certificate. See Box, 139 S. Ct. at 1782 ("[T]he state need not have drawn 'the perfect line,' as long as the 'the line actually drawn [is] a rational' one.") (internal citations omitted).

The evidence is unclear how long natural immunity lasts, and it may last for as little as 90 days.[14] This evidence refutes any claim of arbitrariness on the part of the government. It was not irrational for the government of Puerto Rico to create a policy that does not recognize natural immunity for a health certificate if natural immunity may last such a short period of time. Plaintiffs therefore fail to show that Regulation 138-A is not rationally related to a legitimate government interest or that it is arbitrary, oppressive, unreasonable, and going far beyond what is reasonable for the protection of the public. If a person's natural immunity presents some sort of health risk to becoming vaccinated, there is an opportunity to receive a health exemption from a doctor.[15] Although Plaintiffs may prefer a different policy alternative, the policy decisions that public health officials have made is due some deference, and the court must not engage in policymaking.

[14] Although some evidence was presented that for some persons natural immunity could last for well over three months, insufficient scientific evidence was introduced at the preliminary injunction hearing to support the notion that on average, the government's 90 day window is too narrow.

[15] One flaw in the government's conduct is worth addressing. Dr. Cardona, Defendants' expert in public health, immunizations, infectious diseases, vaccine preventable diseases, and pediatry, and who also serves as an adviser to the Secretary of Health, acknowledged that communication was lacking with regard to the process required to obtain an exemption for the health certificate. For an exemption to be useful, the public must be aware of it.

In conclusion, Plaintiffs fail to carry their burden in showing likelihood of success on the merits for their substantive due process claims, both because the challenged government mandates do not violate the rights Plaintiffs raise, and because the government measures survive scrutiny under the Jacobson standard.

c. Whether EO 75 Violates Ms. Vega's Rights under the Religious Freedom Restoration Act[16]

Plaintiffs also argue that the requirement under EO75 that Ms. Vega verify vaccination status violates the Religious Freedom Restoration Act. ECF No. 7 at 19. Congress enacted RFRA to provide "very broad protection for religious liberty," and the law operates to exempt religious believers from laws of general applicability which nevertheless burden that believer's exercise of religion. Burwell v. Hobby Lobby Stores, Inc., 573 U.S. 682, 694 (2014). To prevail on a RFRA claim, the plaintiff must first establish a prima facie case that the application of the challenged law "substantially burdens a sincere religious exercise." Perrier-Bilbo v. United States, 954 F.3d at 413, 431 (1st Cir. 2020). A plaintiff may sustain a RFRA claim with a "complicity-based objection," whereby the religious adherent believes that compliance with a government mandate obligates them to become complicit in a practice that violates their sincerely held religious beliefs. Little Sisters of the Poor Saint Peter and Paul Home v. Pennsylvania, 140 S. Ct. 2367, 2377 (2020) ("They sincerely believed that human life begins at conception and that, because the challenged methods of contraception risked causing the death of a human embryo, providing those methods of contraception to employees would make the employers complicit in abortion.").

[16] Puerto Rico is a territory of the United States. See Franklin California Tax-Free Trust v. Puerto Rico, 805 F.3d 322, 344 (1st Cir. 2015). Although the Supreme Court "has not had occasion to rule on the matter," it has recognized that that the appellate courts have held that RFRA "remains operative as to the Federal Government and federal territories and possessions." Cutter v. Wilkinson, 544 U.S. 709, 715 n.2 (2005).

Once a plaintiff has established that prima facie case, the burden shifts to the government. Burwell, 573 U.S. at 694–95. The Supreme Court in Burwell v. Hobby Lobby Stores, Inc. explained the process, writing, "If the Government substantially burdens a person's exercise of religion, under the Act that person is entitled to an exemption from the rule unless the Government demonstrates that application of the burden to the person—(1) is in furtherance of a compelling governmental interest; and (2) is the least restrictive means of furthering that compelling governmental interest." Burwell, 573 U.S. at 694–95 (internal quotations omitted).

Ms. Vega succeeds in demonstrating a sincere religious belief in objecting to vaccinations. Ms. Vega testified at the evidentiary hearing that because of her Christian faith she is particularly opposed to the COVID-19 vaccination, explaining that she believes that the vaccine mandates are a fulfillment of "Apocalypse 13 [Chapter 13 of the Book of Revelation of the Bible]" whereby all people will be "marked" in the end times. Hearing, Dec. 7, at 1:44 PM; 2:27 PM.[17] She stated that her "personal and religious interpretation is that we are reaching the last days, and with all of these impositions [her] interpretation is that this is the mark of the Beast." Hearing, Dec. 7, at 2:27 PM. Therefore, Ms. Vega stated that she is religiously opposed to all mandates requiring her to check for proof of vaccination or to ask for a negative COVID-19 test because such requirements are all part of the aforementioned marking process and her compliance makes her an "accomplice of everything." Hearing, Dec. 7, at 1:49–1:52 PM. Therefore, Ms. Vega raises a complicity-based objection to the COVID-19 mandates.

However, Ms. Vega failed to demonstrate a prima facie case of how checking for a negative COVID-19 test violates a sincerely held religious belief. With regard to her personal

[17] Ms. Vega's testimony elaborated her beliefs as follows: "[The Book of Revelation Chapter] 13 emphasizes a lot that we will all be marked, the poor, the rich, slaves, the small, old . . . Because of everything that is happening with the government where we are forced to get vaccinated, we can't go to restaurants or movie theaters those of us who are not vaccinated, just for that the word of God is being complied with." Hearing, Dec. 7, at 1:44 PM.

challenge to the vaccine mandates, Ms. Vega additionally explained that she is also religiously opposed to vaccinations and COVID-19 testing because, as she described, "anything I do with my body that does not comply with God's words . . . when I die I have to report to him, and I have to worry about what I do with my body." Hearing, Dec. 7, at 1:45 PM. She asserted that she believes all vaccinations to be sinful because they "are made with fetuses." Hearing, Dec. 7, at 2:31 PM. Ms. Vega also objects to COVID-19 testing based on introducing an unknown object into her body. Regarding COVID-19 testing, she stated that "We don't know what's inside, how they do it. I understand it is a small wooden stick that they put up in your nose. And I don't know what that contains inside." Hearing, Dec. 7, at 1:48 PM. Upon further questioning by the court, Ms. Vega admitted that she has never asked a physician what the "wooden stick" contains to resolve her uncertainty. Hearing, Dec. 7, at 1:48 PM. Accordingly, Ms. Vega's objection to COVID-19 tests has little to do with religious sincerity as much as with a willful decision not to ascertain the contents of a COVID-19 swab. Unlike vaccines, which Ms. Vega opposes because they may have been made in human fetal cells—a valid religious complicity objection according to Burwell—Ms. Vega offers no sincere religious reason why she cannot ask for proof of a COVID-19 test.

The Defendants argue that allowing business owners like Ms. Vega to require evidence of a COVID-19 test instead of proof of vaccination complies both with the religious exemption required by RFRA and also constitutes a less restrictive means to comply with the vaccine mandate. ECF No. 20 at 13. Defendants' point is well taken. It is already clearly established that stopping the spread of COVID-19 is a compelling government interest. Cuomo, 141 S. Ct. at 67. When it comes to Ms. Vega, she has clearly expressed a prima facie reason why asking for vaccination under her religious beliefs makes her complicit in the distribution of the "mark of the

Beast." However, she fails to demonstrate why COVID-19 tests truly violate a sincerely held religious belief, instead only testifying that she objects without having ascertained the contents of a COVID-19 swab. On this basis alone, Ms. Vega is not being forced to act in a complicit manner with EO75. Ms. Vega does not have to require or accept evidence of vaccination for guests to stay at Hillside Cabin. She can instead only require evidence of a negative COVID-19 test or evidence of a positive COVID-19 infection and proof of recovery in the last three months. As applied to Ms. Vega, EO75 cannot be said to violate Ms. Vega's free exercise rights under RFRA because requiring proof of COVID-19 testing is a less restrictive means of achieving the Puerto Rico government's compelling interest of limiting the spread of COVID-19. Therefore, Ms. Vega fails to show a substantial likelihood of success on that claim.

d. Whether EO 75 and Regulation 138-A Violate the Puerto Rico Constitution

Finally, invoking the supplemental jurisdiction of the federal court, Plaintiffs argue that EO75 violates the Puerto Rico Constitution. ECF No. 7 at 21–31. The Puerto Rico Supreme Court recognizes that "The separation of powers in Puerto Rico is expressly enshrined in Art. I, Sec. 2 of the Constitution of the Commonwealth of Puerto Rico." Colón-Cortés v. Pesquera, 150 D.P.R. 724, 2000 PR Sup. LEXIS 56 (2000). Plaintiffs argue that it is a violation of the separation of powers for the statute that authorizes the governor to act in an emergency, 25 L.P.R.A. § 3650, to grant him the power to enact executive orders like EO75. ECF No. 7 at 22–24. Instead, Plaintiffs urge that regulations like EO75 must be enacted through the Health Department which must act pursuant to the Puerto Rico Uniform Administrative Procedure Act, 3 L.P.R.A. § 9601-9713. ECF No. 7 at 24. Plaintiffs contend that the Uniform Procedure Act contains an emergency rulemaking procedure which would allow the governor to make a

regulation effective immediately subject to the regular rulemaking process. ECF No. 7 at 25 (citing 3 L.P.R.A. § 9623).

The first step of statutory interpretation and discerning the intent of the legislature is to look to the plain language of the statute. If the language is clear, then the job of the court is complete. Bostock v. Clayton County, Georgia, 140 S. Ct. 1731, 1750 (2020) ("This Court has explained many times over many years that, when the meaning of the statute's terms is plain, our job is at an end. The people are entitled to rely on the law as written, without fearing that courts might disregard its plain terms based on some extratextual consideration.").

EO75 invokes 25 L.P.R.A. § 3650, or the "Puerto Rico Public Safety Department Act" as the source of its authority. An examination of the plain language of the statute reveals that the Puerto Rico Legislature did indeed authorize the Governor to issue regulations like EO75 after the Governor declares a state of emergency. Of particular interest is 25 L.P.R.A. § 3650(b)-(c) which provides:

> In emergency or disaster situations, the Governor of Puerto Rico may declare through a proclamation that a state of emergency or disaster exists, as the case may be, in all of the territory of Puerto Rico or part thereof. The Governor, for the duration of such state of emergency or disaster shall have, in addition to any others conferred by other laws, the following powers:
> . . .
> (b) May prescribe, amend, and revoke any regulations as well as issue, amend, and rescind such orders as deemed convenient which shall be in effect for the duration of the state of emergency or disaster. Regulations prescribed or orders issued during a state of emergency or disaster shall have force of law for the duration of the state of emergency or disaster.
> (c) May render effective any state regulations, orders, plans, or measures for emergency or disaster situations or modify them at his discretion.

25 L.P.R.A. § 3650(b)-(c). A reading of the statute demonstrates that the Puerto Rico Legislature did indeed act to grant the Governor the power to issue, amend, and rescind both "regulations" and "orders" during a state of emergency. 25 L.P.R.A. § 3650(b). These "[r]egulations

prescribed or orders" are to have force of law during the duration of the emergency. This delegation is made directly to the Governor, and not to the Secretary of Health, and the plain language makes no mention of the Puerto Rico Uniform Administrative Procedure Act—emergency procedure or otherwise. It is therefore a reasonable construction of the statute that under a state of emergency, the Governor does not have to act through the Uniform Administrative Procedure Act to promulgate regulations pertaining to the emergency at issue. The plain language is clear that the Puerto Rico Legislature intended to convey the power to the Governor to create regulations like EO75.

Along these same lines, Plaintiffs argue that EO75 lacks the statutory authority to include the threat of criminal penalties for non-compliance with the Executive Order. ECF No. 7 at 30–31. Plaintiffs assert that neither 25 L.P.R.A. § 3650 nor the Health Department Act provides for a criminal penalty. ECF No. 7 at 30. Once again, the language of the statute at issue is instructive. Section 14 of EO75 describes the penalties for failing to comply with EO75 and makes reference to 25 L.P.R.A. § 3654 of the Puerto Rico Department of Public Safety Act. Subsection (d) of 25 L.P.R.A. § 3654 provides,

> Any person who commits any of the following acts shall be punished by imprisonment for a term not to exceed six (6) months or a fine not to exceed five thousand dollars ($5,000), or both penalties at the discretion of the court:
> . . .
> (d) Persisting in carrying out any activity that endangers his life or the lives of other persons, after having been warned by the authorities once a hurricane warning has been issued or a state of emergency has been declared by the pertinent authorities, or while a state of emergency declared by the Governor of Puerto Rico through an Executive Order is in effect.

25 L.P.R.A. § 3654(d). On the basis of this statute alone, it is clear that the Legislature did provide for criminal penalties when a person acts to endanger his life or the lives of other persons while a state of emergency has been declared by the Governor of Puerto Rico through an

Executive Order. Plaintiffs seem to argue that 25 L.P.R.A. § 3654 is inapplicable because "noncompliance with an [executive order] is not included among these." ECF No. 7 at 30. However, as discussed above, the Governor of Puerto Rico has been empowered to issue executive orders during a state of emergency; therefore disobedience to an executive order that seeks to protect human life while a state of emergency is in effect is one sort of violation that would logically fall under the ambit of this statute.

Nevertheless, Plaintiffs argue that it cannot be a "sound construction" of 25 L.P.R.A. § 3650 to "say that the governor may issue any executive order he deems "convenient" with whatever content or impact upon fundamental rights he decides . . . ." ECF No. 7 at 24. These arguments more directly aim at the delegation doctrine rather than whether the Governor has been granted this power at all by the Legislature. Accordingly, Plaintiffs assert at length that if 25 L.P.R.A. § 3650 does indeed grant the authority to the Governor to issue executive orders like EO75, then such a delegation is an unconstitutional violation of the non-delegation doctrine. ECF No. 7 at 25–30. They argue that granting the Governor the power to make regulations and orders as he deems "convenient" is too broad of a guideline to constitute an intelligible principle. ECF No. 28–29.

In support of their argument Plaintiffs cite Gundy v. United States: "[A] statutory delegation is constitutional as long as Congress lay[s] down by legislative act an intelligible principle to which the person or body authorized to [exercise the delegated authority] is directed to conform." Gundy v. United States, 139 S. Ct. 2116, 2123 (2019) (internal citations omitted). In that case, however, the Supreme Court declined to strike down the Congressional grant of authority on grounds of the delegation doctrine. Indeed, in his concurrence, Justice Samuel Alito acknowledged that prevailing on a non-delegation doctrine argument has become an uphill

endeavor. Gundy, 139 S. Ct. at 2130–31 (Alito, J. concurring) ("Nevertheless, since 1935, the Court has uniformly rejected nondelegation arguments and has upheld provisions that authorized agencies to adopt important rules pursuant to extraordinarily capacious standards.").

Furthermore, insofar as the delegation doctrine applies in Puerto Rico, the Supreme Court has cautioned that Puerto Rico's unique judicial status merits a strong degree of "rigid deference" to local courts' interpretations of the Puerto Rico Constitution as applied to local statute. Corporación Insular de Seguros v. García, 680 F. Supp. 476, 482 (D.P.R. 1988) ("The factor, by now clear, is that issues of Puerto Rico law, including constitutional interpretations of local statutes, require a "rigid deference" to the actions of local courts.") (citing Díaz v. González, 261 U.S. 102, 105–106 (1923); Bonet v. Texas Co. of Puerto Rico, Inc., 308 U.S. 463, 470–471 (1940) ("For over sixty years this Court has consistently recognized the deference due interpretations of local law by (Puerto Rico) courts unless they appeared clearly wrong . . . . We now repeat once more the admonition.").

Defendants point out in their brief, with Plaintiffs' correctly acknowledging the court's expressions as dicta, that the Court of First Instance, San Juan Part, of the Commonwealth of Puerto Rico, in Amadeo et al. v. Pierluisi-Urrutia et al., wrote that, "In what is relevant to this case, Article 6.10 of the Puerto Rico Public Safety Department Act, Act No. 20-207 [25 L.P.R.A. § 3650], constitutes a clear example in which the Legislative Assembly conferred to the Governor ample faculties to act in protection of public interest in cases of emergency." ECF No. 18-1 at 17. Such a finding is consistent with the deference federal courts have so far granted to legislative decisions on managing the pandemic. As the Supreme Court held in Jacobson, "the police power of a state must be held to embrace, at least, such reasonable regulations established directly by legislative enactments as will protect the public health and the public safety."

Jacobson, 197 U.S. at 25. Indeed, the court in Jacobson recognized that in such emergency situations that this police power might be "exercised directly by the legislature, or by a local body acting under its authority." See Jacobson, 197 U.S. at 38. Furthermore, as declared by Justice John Roberts' concurrence in S. Bay United Pentecostal Church, "[t]he precise question of when restrictions on particular social activities should be lifted during the pandemic is a dynamic and fact-intensive matter subject to reasonable disagreement. Our Constitution principally entrusts the safety and the health of the people to the politically accountable officials of the States." S. Bay United Pentecostal Church, 140 S. Ct. at 1613 (Roberts, C.J., concurring).

The guidelines provided by the Legislature are broad. The Puerto Rico Public Safety Department Act merely grants the Governor the power to issue regulations and orders "as deemed convenient" during a state of emergency and he is allowed to "modify them at his discretion." 25 L.P.R.A. § 3650(b)-(c). Perhaps in normal times such guidelines would violate an "intelligible principle." However, the public policy rationale of the Legislature's delegation of Power to the Governor during a public emergency is clear. In times of emergency, rather than relying upon the Legislature or a lengthy notice and comment period—which may take significantly more time than what is required to address the emergency—the Puerto Rico Legislature has conveyed that power to the Governor so that he can act quickly. Plaintiffs argue that because 25 L.P.R.A. § 3650 was enacted to "reform Puerto Rico's public security system . . . to combat criminality and violence in Puerto Rico", the law "cannot be construed to authorize the [G]overnor to declare an emergency of a completely different nature, such as learning to grapple with COVID-19." ECF No. 23–24. However, no legislature can precisely predict the contours of every state of emergency. Therefore, it would be unreasonable to expect a legislature to list all the potential emergencies imaginable before they could empower the governor to quickly take

action in an emergency. It would also be impractical to expect a legislature, as Plaintiffs assert, to define an intelligible principle with any more specificity for states of emergency because such guidelines may differ greatly depending on the state of emergency. The guidelines or "intelligible principle" may be dramatically different during a hurricane or flooding than during a pandemic or widespread civil unrest.

A word of caution, however, is in order. The grant of power to the Governor must be closely tied to the scientific and factual evidence triggering the state of emergency. Where "local officials are actively shaping their response to changing facts on the ground" and "[w]hen those officials undertake[] to act in areas fraught with medical and scientific uncertainties, their latitude must be especially broad." S. Bay United Pentecostal Church, 140 S. Ct. at 1613 (Roberts, C.J., concurring). We now know much more about COVID-19 and the virus that causes it than when S. Bay Pentecostal Church was decided in May 2020. As more knowledge is gained about the pandemic, scientific uncertainty may become less of a justification for expansive government powers and the curtailing of rights.

In conclusion, while admittedly broad, concerns of deference to local constitutional interpretation, public policy underlying the delegation of authority to the Governor, and the scientific evidence underlying the continued public emergency in Puerto Rico lead to the conclusion that the Legislature's statutory grant of authority to the Governor does not violate the delegation doctrine. Plaintiffs therefore have not shown a substantial likelihood of success on the merits based on the Puerto Rico constitutional challenge to EO75.

B. Irreparable Injury

Turning now to the second prong, if a court finds that the moving party fails to show it is likely to succeed on the merits, it may, within its discretion deny relief without addressing the

remaining three factors. New Comm. Wireless Servs., Inc. v. SprintCom, Inc., 287 F.3d 1, 9 (1st Cir. 2002). Nevertheless, the court will weigh whether the Plaintiffs' face any irreparable harm. The First Circuit has held that "infringements of free speech, association, privacy or other rights as to which temporary deprivation is viewed of such qualitative importance as to be irremediable by any subsequent relief." Pub. Serv. Co. of New Hampshire v. West Newbury, 835 F.2d 380, 382 (1st Cir. 1987).

As written, EO75 does not force any violation of bodily integrity, medical choice, or medical privacy for the Plaintiffs who object to having to undergo a test to engage in public activities. As discussed above, Section 10 of EO75 requires restaurants, bars, hair salons, hotels, and many other public places to check either proof of vaccination or proof of a COVID-19 test showing a negative result or of a positive result in the last three months accompanied by evidence of recovery. Businesses also have the option of operating at 50 percent capacity, which would allow it to serve customers without asking for proof of vaccination or a negative COVID-19 test. Tropical Chill has opted for this practice to avoid violating its customers' right to medical privacy. By way of this option, Plaintiffs can avoid violations to medical privacy rights, bodily integrity, and medical choice by restricting capacity to 50 percent in their own business, or by choosing to frequent businesses that only allow 50 percent capacity.[18] Yet, even if businesses did not have the option of operating at 50 percent capacity, and all visitors were required to show proof of vaccination or one of the required COVID-19 test results, the Plaintiffs' harm still cannot be deemed "irreparable" for the reasons explained below.

[18] The Plaintiffs' tendered "Second Rule 15(d) Supplemental Pleading" alleges that the Governor's current executive orders, specifically Executive Order 2021-081, now eliminate the 50 percent capacity option for affected business, meaning that the covered businesses must operate at 50 percent capacity and check vaccination and COVID-19 test status. ECF No. 95-1 at 2–3.

a. Plaintiffs' Harm to Medical Privacy

Regarding Plaintiffs' harm to their medical privacy, the Puerto Rico government has undoubtedly placed a significant burden on Plaintiffs' social lives for refusing to relinquish some measure of medical privacy. EO75 places non-trivial social consequences on Plaintiffs' decision not to be vaccinated or their refusal to present a negative COVID-19 test. Plaintiffs cannot attend restaurants, concerts, bars, hotels, barber shops, or hair salons unless they allow covered businesses to invade their medical privacy. However, the invasion in this case is slight, and they are not being asked to open the entirety of their medical records to public scrutiny. Plaintiffs are only obligated to show either a proof of COVID-19 vaccination, proof of a negative COVID-19 test, or proof of a positive COVID-19 test within the last three months accompanied by proof of recovery. On that limited basis alone, it cannot be said that Plaintiffs' harm to their medical privacy is of "such qualitative importance as to be irremediable by any subsequent relief." Pub. Serv. Co. of New Hampshire, 835 F.2d at 382.

b. Plaintiffs' Harm to Personal Autonomy, Bodily Integrity, and Medical Choice

Turning to Plaintiffs' claimed violations of personal autonomy, bodily integrity, and medical choice, Plaintiffs are undoubtably subject to harm, but it is not irreparable. Plaintiffs are not being forced to accept vaccination by EO75—the permanent introduction of substance into a person's body which is impossible to withdraw. Forced vaccination would represent a harm to bodily integrity and medical choice that is that clearly irreparable. However, COVID-19 testing is much less intrusive and results only in a temporary invasion of the body. Businesses continue to have the option of requiring a negative COVID-19 test rather than solely accepting proof of vaccination.

c. Plaintiffs' Economic Harm

Finally, Plaintiffs do not face irreparable economic harm. Generally, economic harm is not irreparable to warrant protection under a preliminary injunction because "traditional economic damages can be remedied by compensatory awards, and thus do not rise to the level of being irreparable." Puerto Rico Hosp. Supply, Inc. v. Boston Scientific Corp., 426 F.3d 503, 507 (1st Cir. 2005). The only exception may be when losses are so large that they may be irreparable, as when "the potential economic loss is so great as to threaten the existence of the movant's business." Vaquería Tres Monjitas, 587 F.3d at 485 (internal citations omitted).

Tropical Chill demonstrated that its economic losses under the mandates are not trivial. Since the mandates went into effect, Mr. Vega testified that Tropical Chill suffered a loss in revenue of almost 20 percent. Hearing, Dec. 6, at 9:30 AM. Additionally, Tropical Chill is currently operating at a loss, Mr. Vega has not collected a salary in the month, and the stores have cut employee hours. Mr. Vega estimated that Tropical Chill would only be able to operate under the restrictions for 30 to 60 days before the losses would result in employee layoffs. However, Mr. Vega did not testify that Tropical Chill is facing economic failure or bankruptcy. Therefore, although Tropical Chill's losses are significant, they do not rise to the level of irreparable harm that would require a lesser showing of likelihood of success. The type of harm that Tropical Chill is suffering is the type of harm that is traditionally remedied through compensatory damages, not prospective injunctive relief.

Ms. Llenza faces a far graver risk of irreparable economic harm because of her decision not to get vaccinated because she might not be able to secure employment in her chosen fields without a health certificate. Ms. Llenza, however, failed to provide convincing evidence that the temporary deprivation to her job prospects is of such a qualitative importance to be irreparable.

Ms. Llenza was last employed as a field inspector for a private contractor in a local disaster relief program in Puerto Rico after Hurricane María. She worked in this position from February until April 2018. Since then, Ms. Llenza was unemployed and pursued training to advance her professional career. Despite that training, Ms. Llenza's explanation for not working since 2018 was because that she was "doing other things." Hearing, Dec. 8, at 10:05 AM. Ms. Llenza became certified as a "Professional Food Manager" in December 2019, but she did not secure a health certificate or seek work in food preparation immediately after being certified in December 2019. Exhibit 41. Instead, Ms. Llenza testified that she only began searching for work at the beginning of 2021.

If Ms. Llenza was facing irreparable harm from her unemployment, that harm would have long ago manifested itself during the lengthy period she was out of work before Regulation 138-A was issued. Ms. Llenza could have secured a health certificate and searched for employment between April 2018 and the beginning of 2021, but she did not. Ms. Llenza also could have secured a health certificate when she began looking for work in early 2021, long before the promulgation of Regulation 138-A in August 2021, but she also failed to do so. For the vast majority of the time that Ms. Llenza was out of work since April 2018, she was unemployed not because of her inability to get a health certificate under Regulation 138-A, but rather for other, unrelated reasons. Such significant delay in looking for work and in seeking a health certificate does not indicate a person who faces irreparable harm from unemployment. Ms. Llenza has been unemployed for nearly four years for reasons unrelated to Regulation 138-A. She therefore fails to show how her continued inability to get a health certificate because she chooses to remain unvaccinated is an irreparable harm.

C. Balance of the Equities

The third prong of the preliminary injunction inquiry requires a balancing of the equities which examines "he hardship that will befall the nonmovant if the injunction issues contrasted with the hardship that will befall the movant if the injunction does not issue . . ." Mercado-Salinas v. Bart Enterprises International, Ltd., 671 F.3d 12, 19 (1st Cir. 2011).

The movants, Plaintiffs, have made their hardships clear while facing the requirements of EO75 and Regulation 138-A. If the court does not issue a preliminary injunction Tropical Chill will continue to suffer financial losses under the Governor's mandates regulating vaccine and COVID-19 checks for businesses. Mr. Matos and Ms. Llenza will have to continue to expend time during the week to secure COVID-19 tests to be able to work or to continue to seek employment. Mr. Matos, Ms. Llenza, and Ms. Vega—if they so choose to attend restaurants, bars, hotels, and other places that check for vaccine status or negative test—will have to relinquish a measure of their liberty interests in medical choice by submitting to a COVID-19 test and will be required to disclose matters of medical privacy by demonstrating evidence of that test. Ms. Vega will also be required to ask, at very least, for evidence of a negative COVID-19 test for guests to stay at her short-term rental. To obtain a health certificate to work in her chosen fields, Ms. Llenza must either submit to vaccination or will have to present evidence from a physician of a medical reason for her ineligibility for vaccination. There is no doubt that as applied, EO75 and Regulation 138-A can burden important liberty or property interests held by the Plaintiffs, even if that burden is rationally related to a legitimate government interest.

However, on balance, the scientific evidence presented at the evidentiary hearing shows that the hardship to the Defendants is greater if a preliminary injunction were issued. The Governor of Puerto Rico and the Puerto Rico Health Department have issued these regulations to

prevent the spread of the COVID-19 virus and its attendant adverse health effects, which could include hospitalizations and deaths. At this time, evidence shows that continued growth of positive cases will result in more hospitalizations, ICU referrals, and deaths in Puerto Rico. This correlation means that uncontrolled spread poses a potential risk of overwhelming the health system of Puerto Rico. A collapse of the public health system would be catastrophic for Puerto Rico and a great risk to human life, and the weight of the hardship at this time outweighs the burden on the Plaintiffs' liberty or property interests.

D. Public Interest

The analysis of the balancing of the equities has a strong bearing on the public interest. Without a doubt, the public has an interest in returning to a state of normality in which their rights are not infringed by the current state of emergency. Granting the preliminary injunction could very well provide some relief from the lengthy state of emergency under which Puerto Rico residents have suffered patiently. However, another public interest is at stake because although Plaintiffs may be willing to shed the current restrictions in favor of freeing their currently burdened liberty and property interests, such a result "can sicken and even kill many others who did not consent to that trade-off." See Cassell v. Snyders, 990 F.3d 539, 550 (7th Cir. 2021). In such a way, the public also certainly has an interest in preventing the collapse of the health system in Puerto Rico. As discussed at length above, the evidence does not yet point to a significant reduction in the risk of that outcome, and this prong must also weigh in favor of denying prospective relief.

Nevertheless, the analysis applied to the balance of the equities and the public interest may change over time. As of December 10, 2021, the death rate in Puerto Rico was 0.1 per 100,000—an extremely small number. Exhibit 61. When asked about that number, Defendants'

expert epidemiologist, Dr. Marzán acknowledged that number was true but retorted "It's a low rate, but it is a preventable death." Hearing, Dec. 13, at 3:21 PM. A functioning society must accept some measure of risk to human life to enjoy the benefits of a modern existence. The question is where to draw the balance. The government is correct that in the past, and at this point, the threat of COVID-19 presents an unacceptable risk to human life and presents a risk of overwhelming the health system in Puerto Rico. However, over time, the SARS-CoV-2 virus may become less lethal, especially as vaccination increases, new treatments are developed, and the virus mutates. Such a future is what science, public health, and the patience of residents of Puerto Rico have striven to achieve. As the virus becomes less likely to overwhelm the capacities of public health, whether that be from natural evolution, vaccination, or natural immunity, or new treatments, the lines balancing the equities and supporting the public interest may have to be redrawn.

V. CONCLUSION

In conclusion, Plaintiffs have not shown a substantial likelihood of success on the merits, nor have they demonstrated a likelihood of irreparable harm absent interim relief. The balancing of the equities and the public interest also do not weigh in favor of granting the motion for preliminary injunction. After considering the arguments of the parties, the pertinent authorities, and the evidence produced at the evidentiary hearing, Plaintiffs' Motion for Preliminary Injunction should be DENIED.

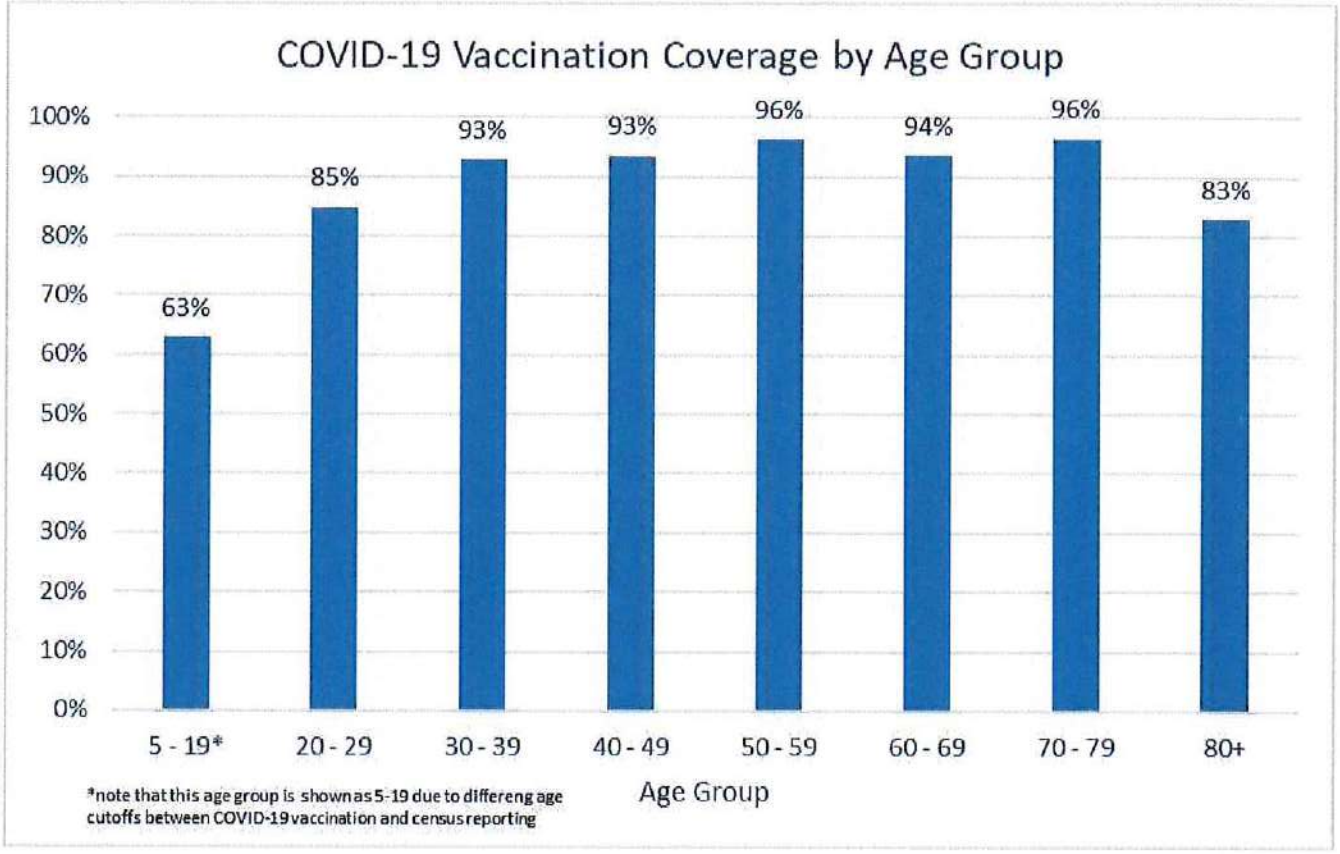
The parties have fourteen (14) days to file any objections to this report and recommendation unless otherwise ordered by the court. Failure to file the same within the specified time waives the right to object to this report and recommendation. Fed. R. Civ. P. 72(b)(2); Fed. R. Civ. P. 6(c)(1)(B); Local Rule 72(d); see also 28 U.S.C. § 636(b)(1); Henley

Drilling Co. v. McGee, 36 F.3d 143, 150–51 (1st Cir. 1994); United States v. Valencia, 792 F.2d 4 (1st Cir. 1986).

IT IS SO RECOMMENDED.

In San Juan, Puerto Rico, this 17th day of January, 2022.

s/Marcos E. López
U.S. Magistrate Judge



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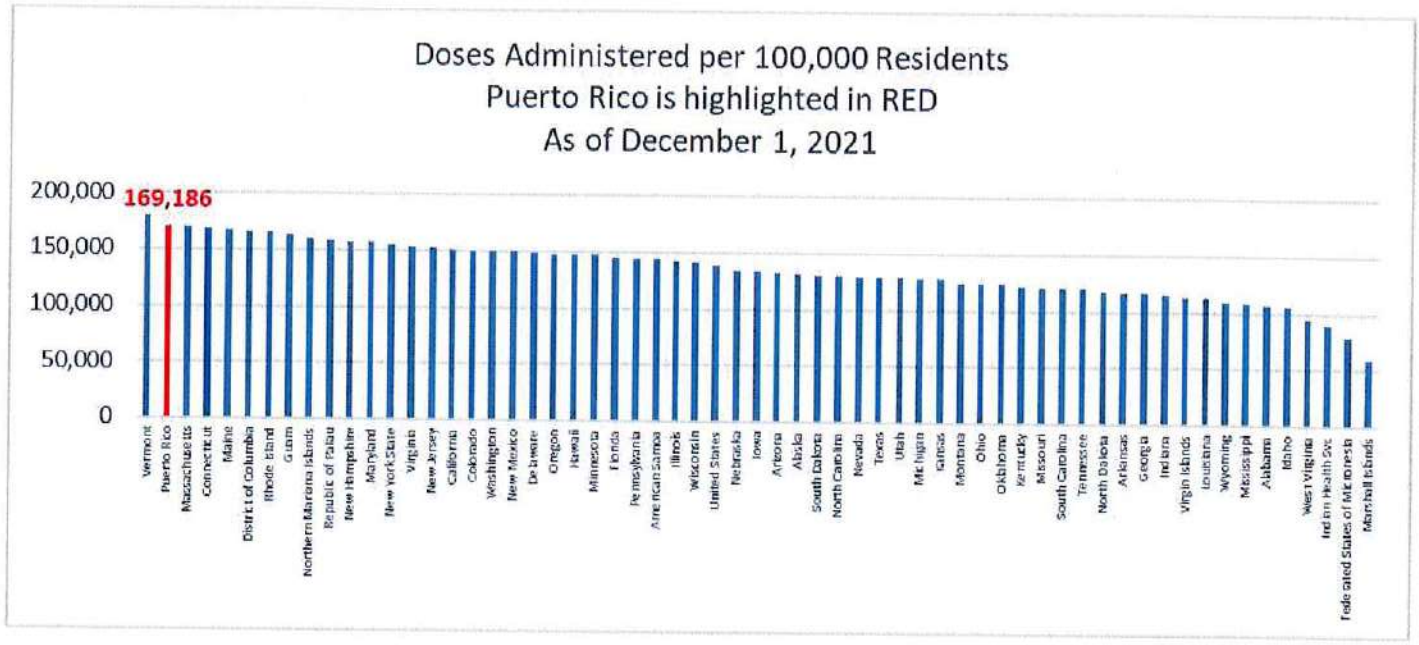
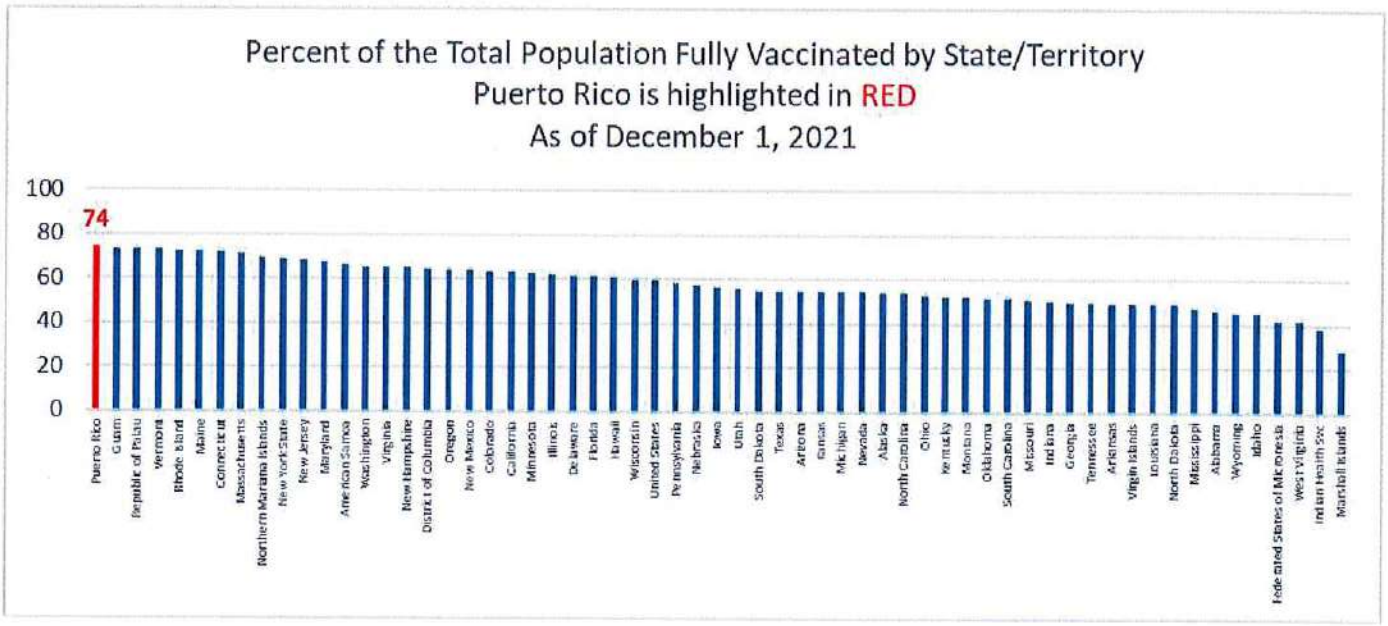
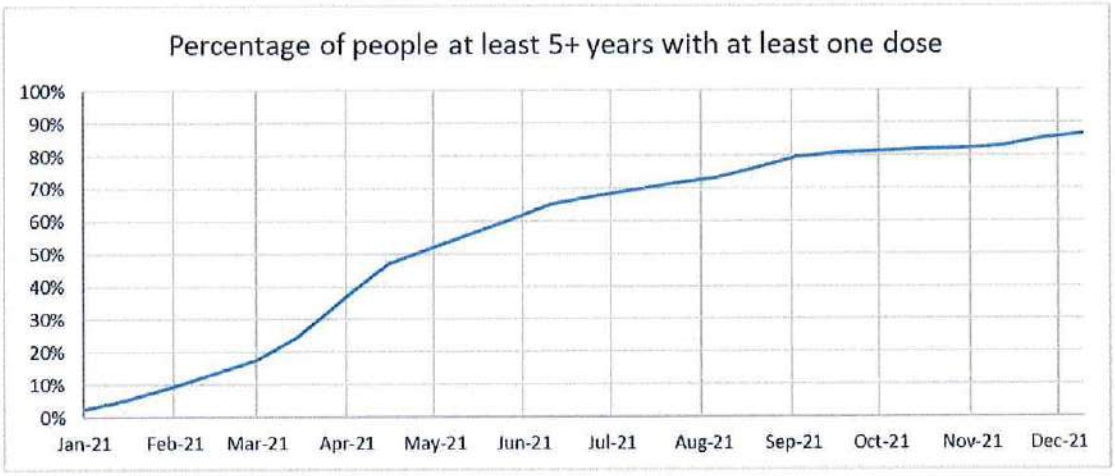
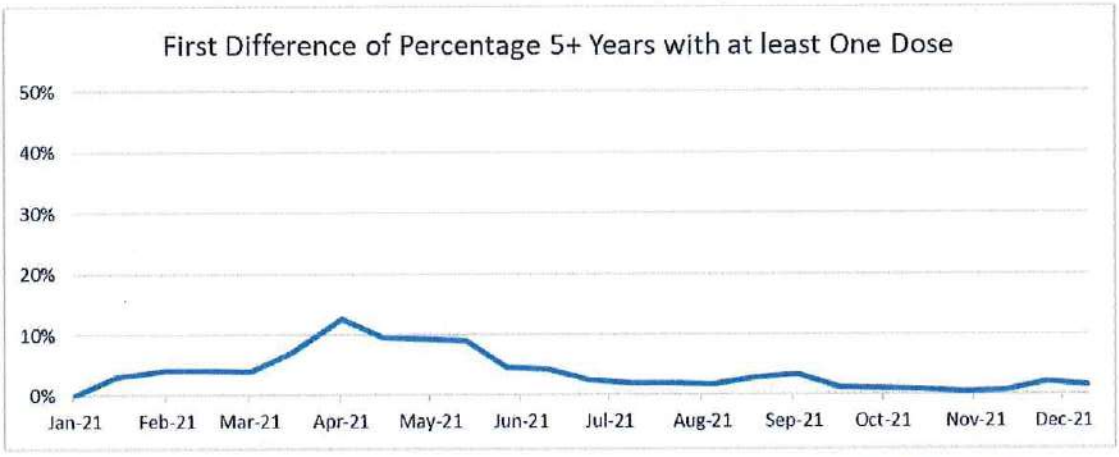


Data source: CDC, COVID-19 Vaccinations in the United States (View: Total Dose, Show: Administered, Metric: Rate per 100,000, Population: Total Population), *Data Table for COVID-19 Vaccinations in the United States (Doses Administered per 100k by State where Administered)*, https://covid.cdc.gov/covid-data-tracker/#vaccinations_vacc-total-admin-rate-total



Data source: CDC, COVID-19 Vaccinations in the United States (View: People, Show: Fully Vaccinated, Metric: % of Population, Population: Total Population), *Data Table for COVID-19 Vaccinations in the United States (Percent of Total Pop Fully Vaccinated by State of Residence* https://covid.cdc.gov/covid-data-tracker/#vaccinations_vacc-people-fully-percent-total





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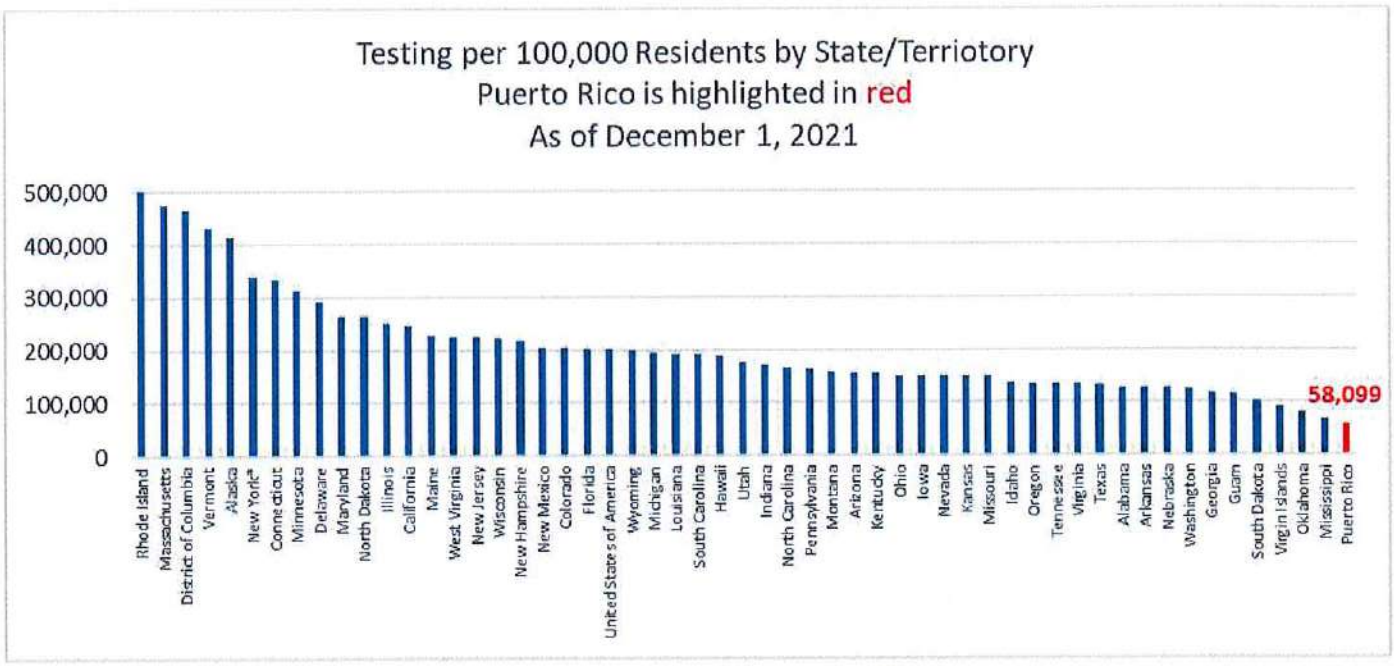
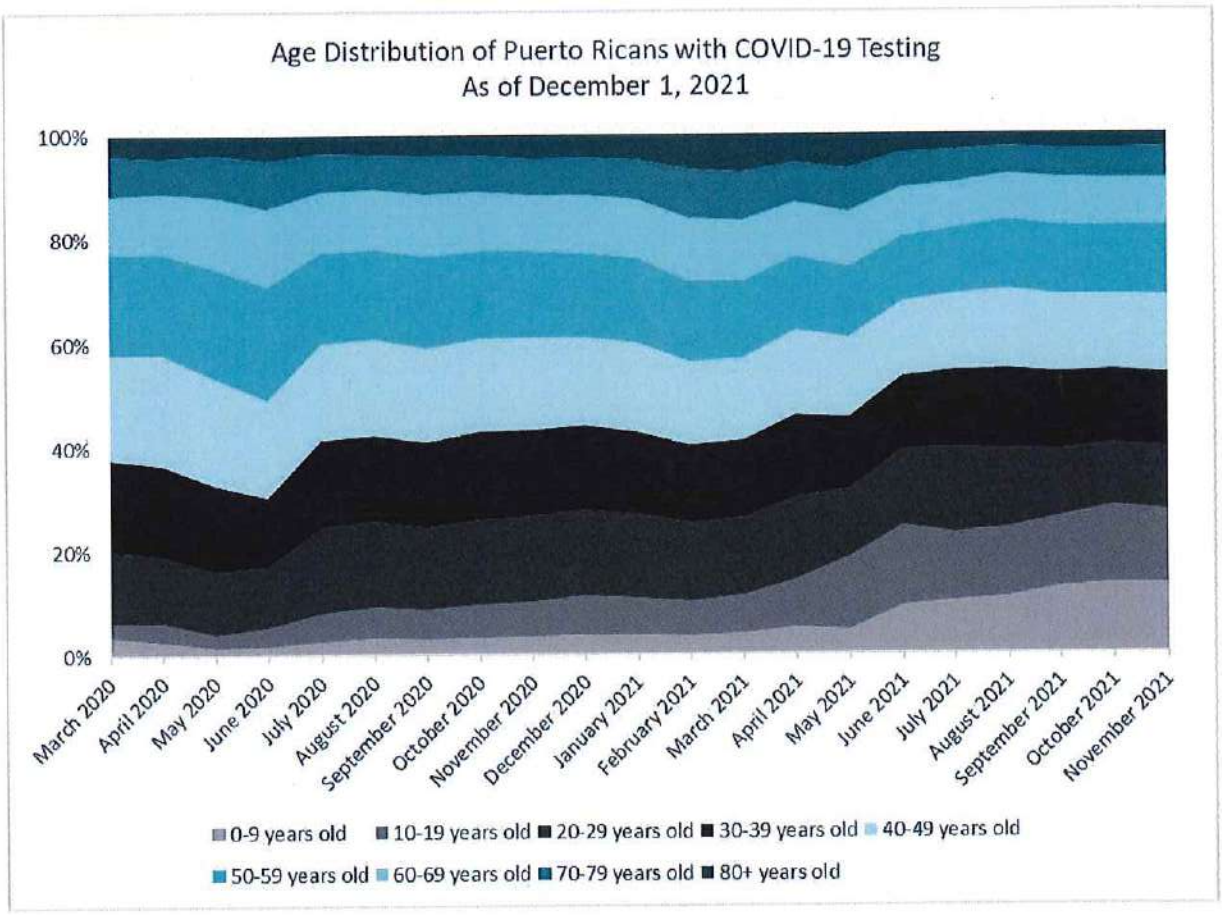


Data Source: CDC, United States COVID-19 Cases, Deaths, and Laboratory Testing (NAATs) by State, Territory, and Jurisdiction (View: Tests Performed, Time period: All Time, Metric: Rate per 100,000), Data Table for Cumulative COVID-19 Nucleic Acid Amplification Tests (NAATs) Performed per 100k by State/Territory, https://covid.cdc.gov/covid-data-tracker/#cases_testsper100k



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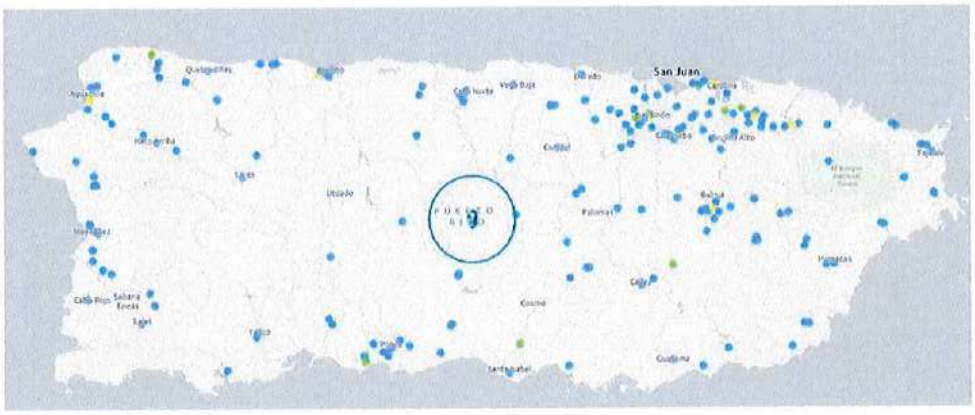
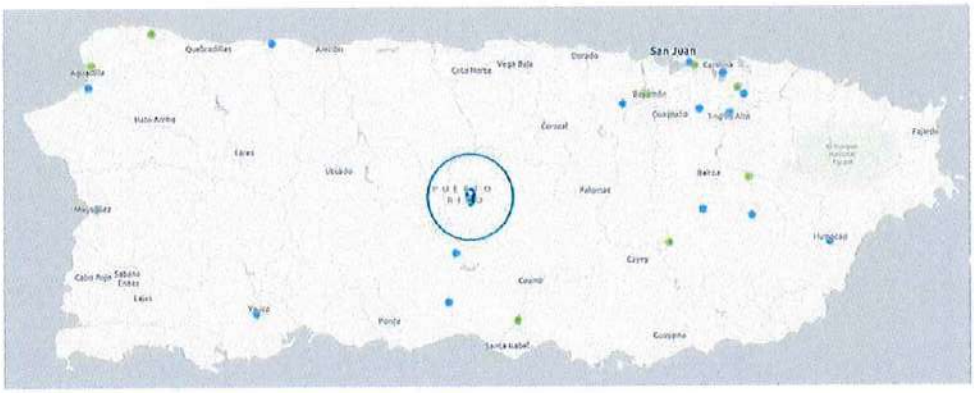
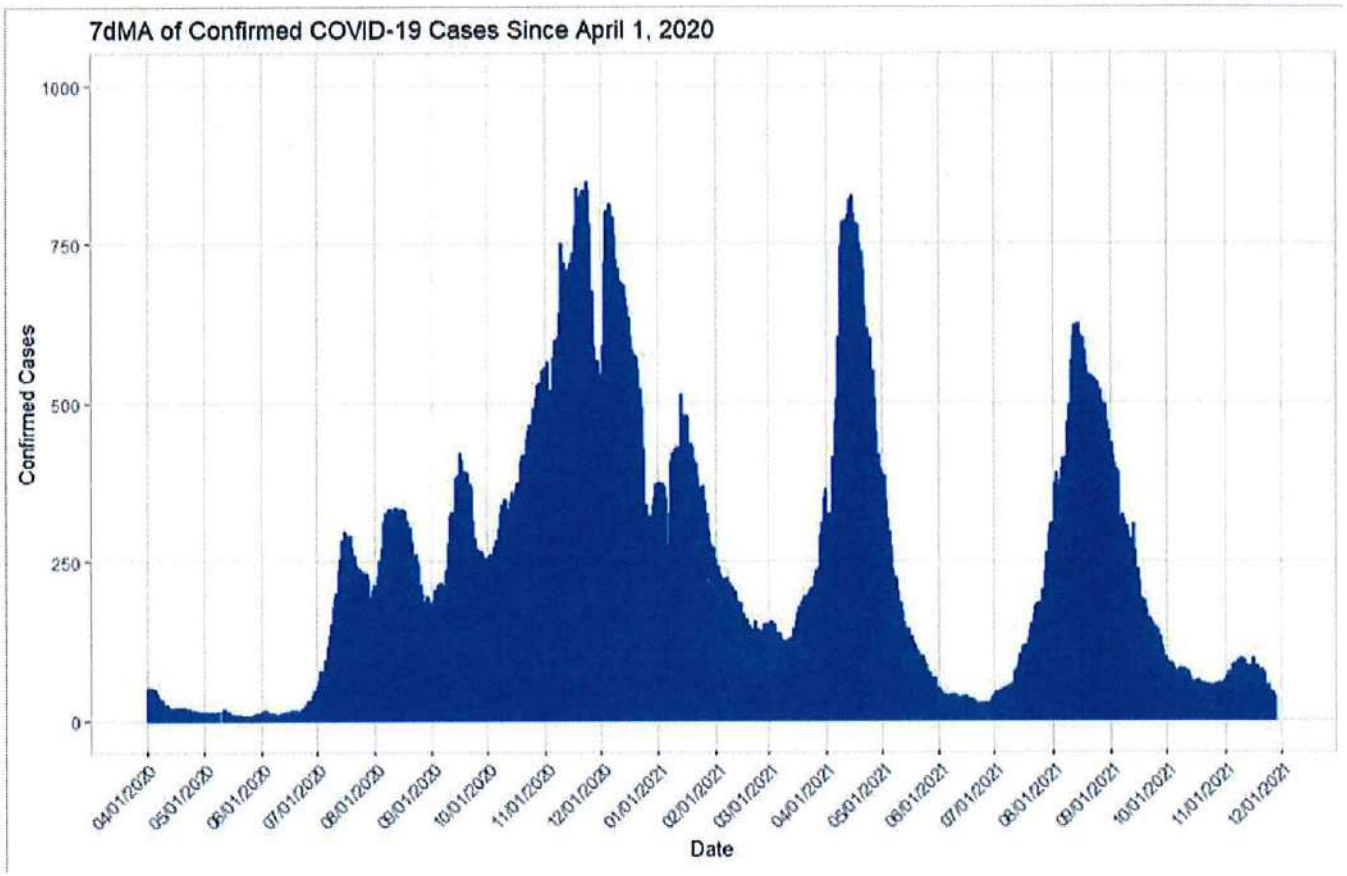


All Testing Sites



Same Day Results – Testing Sites

GISCorps COVID-19 Testing Sites Locator. Accessed September 12, 2021., https://www.arcgis.com/apps/webappviewer/index.html?id=2ec47819f57c40598a4eaf45bf9e0d16



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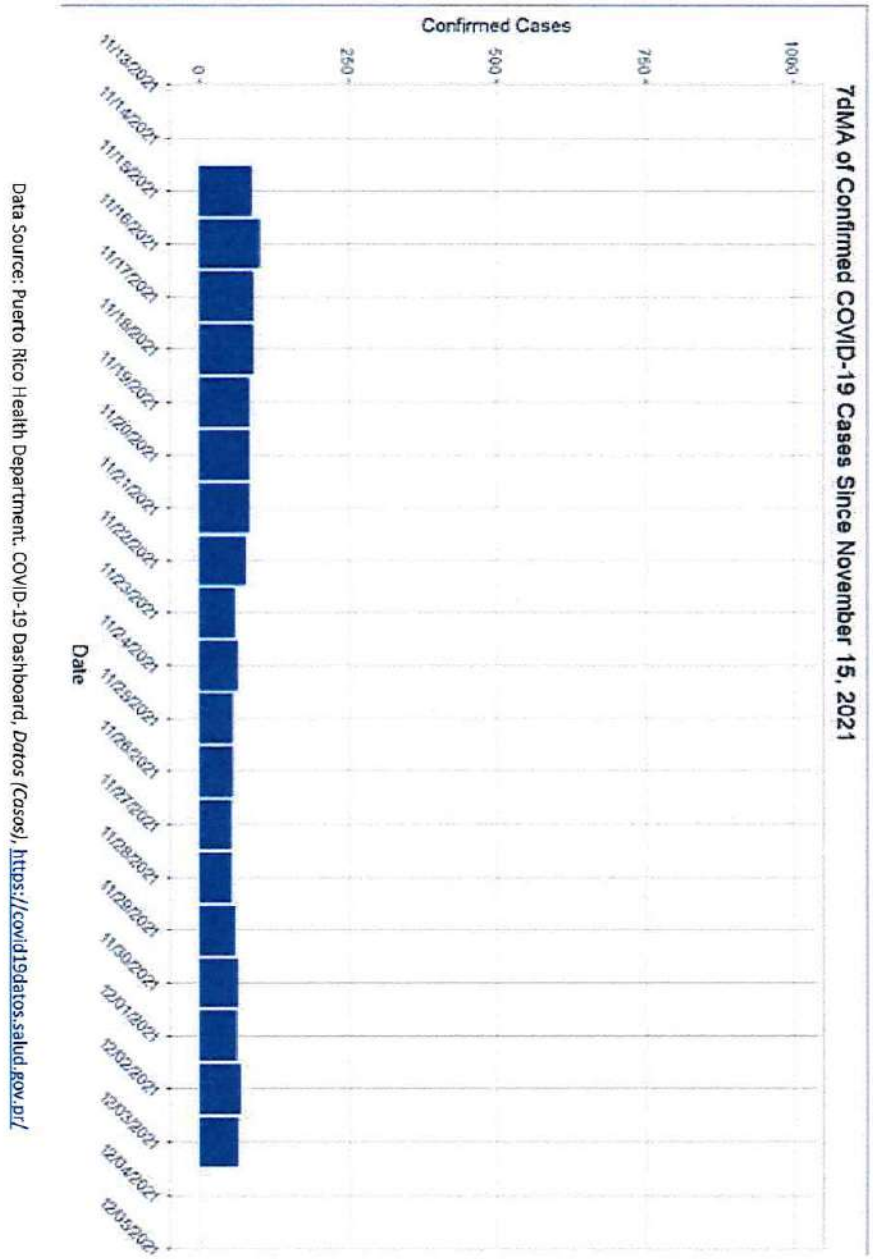

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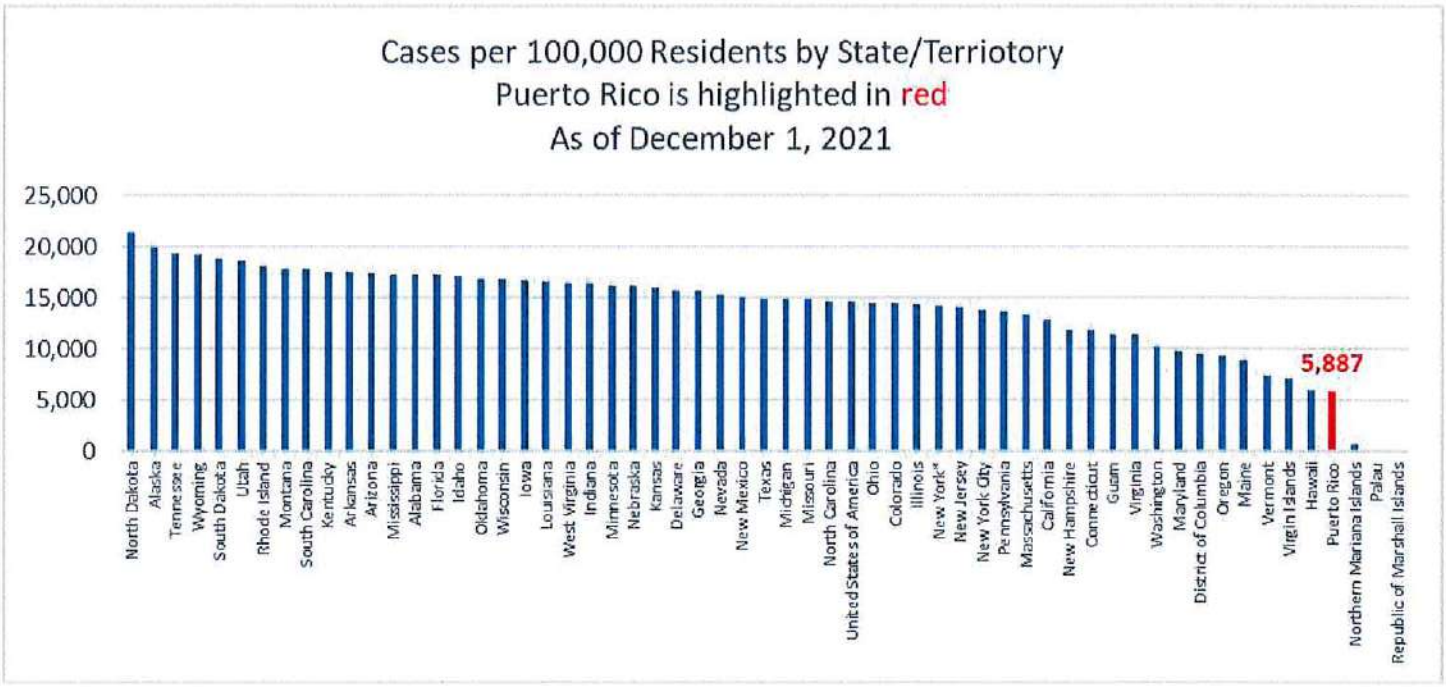

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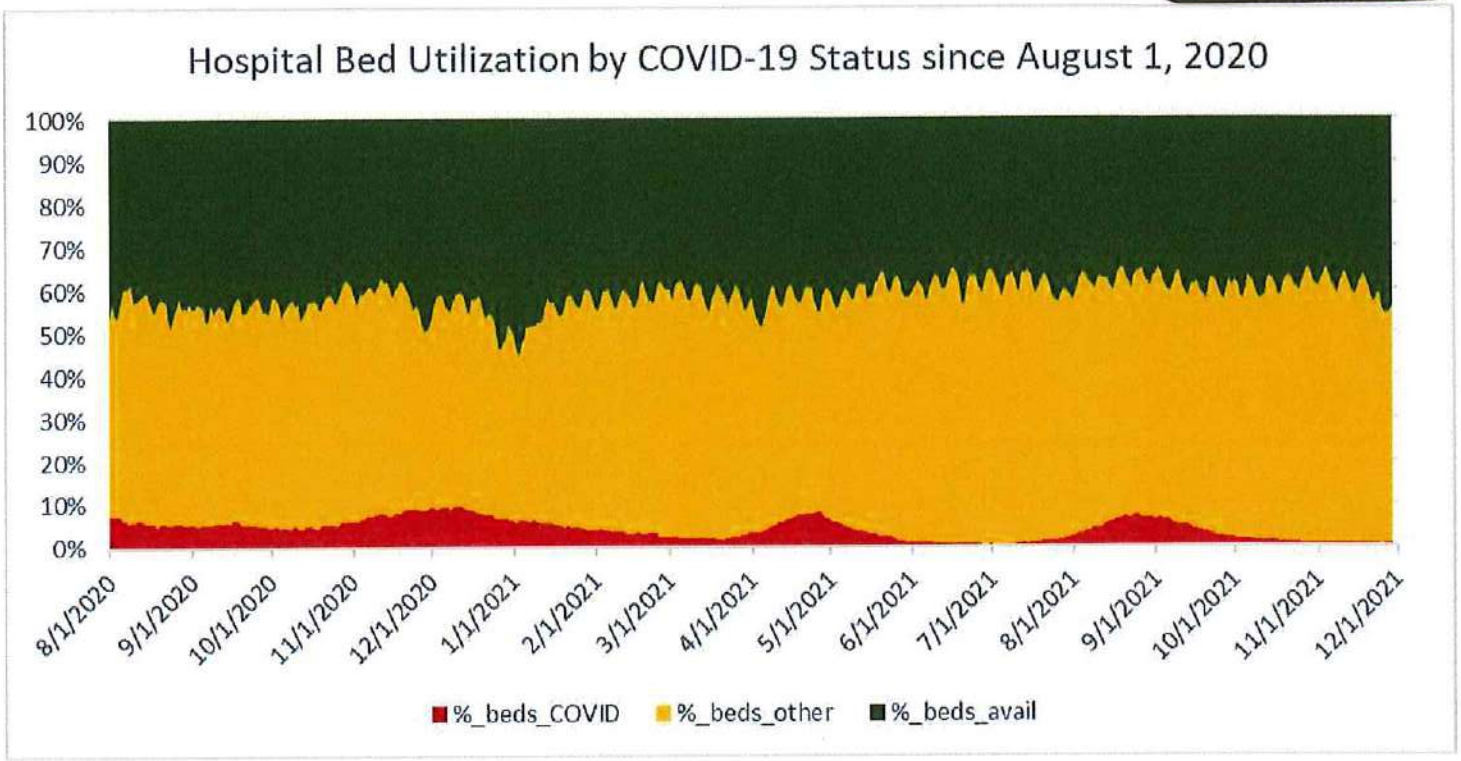

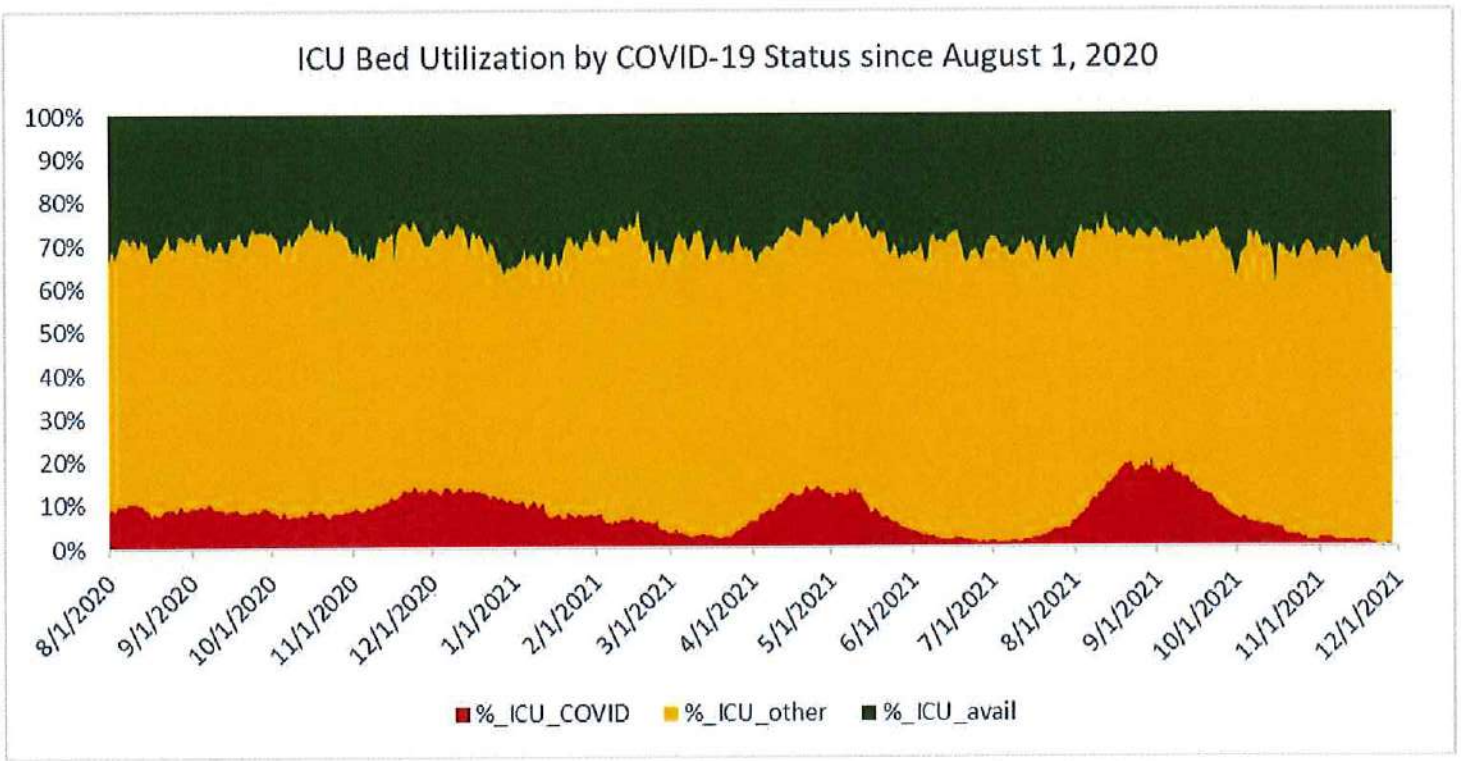

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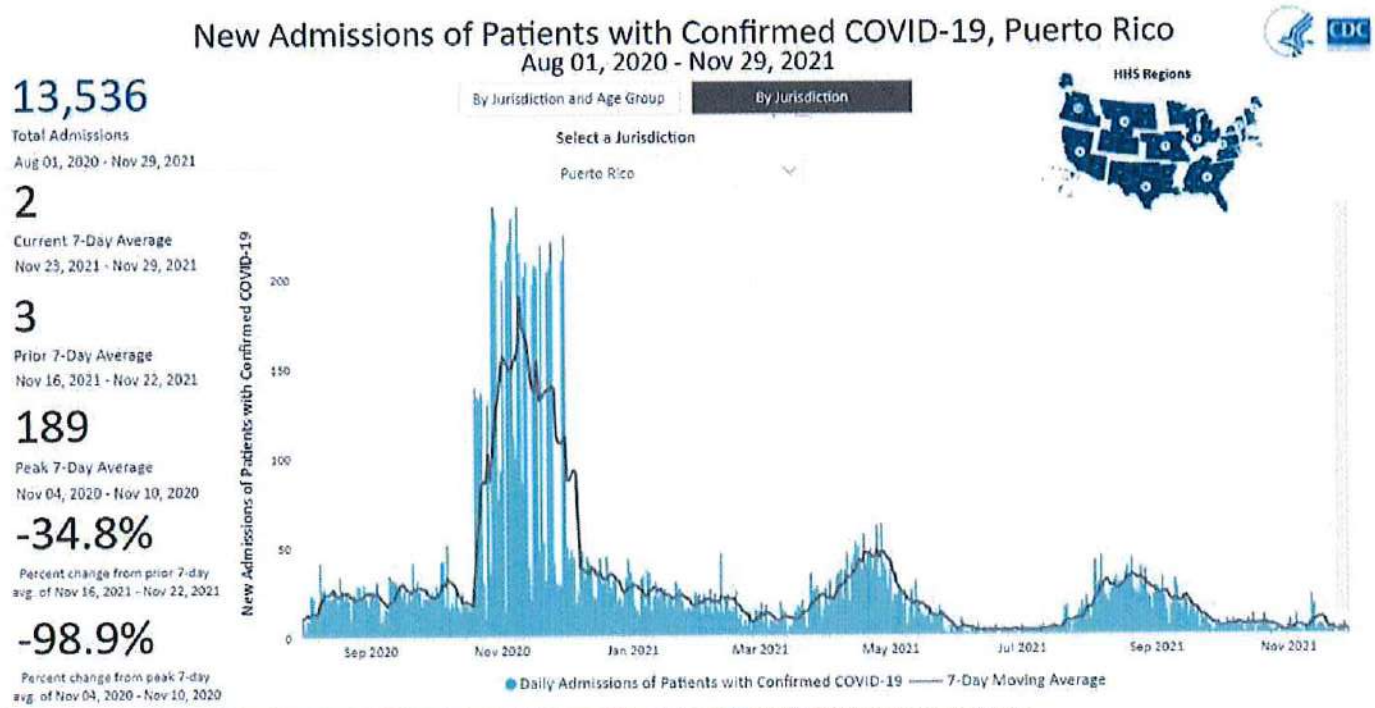
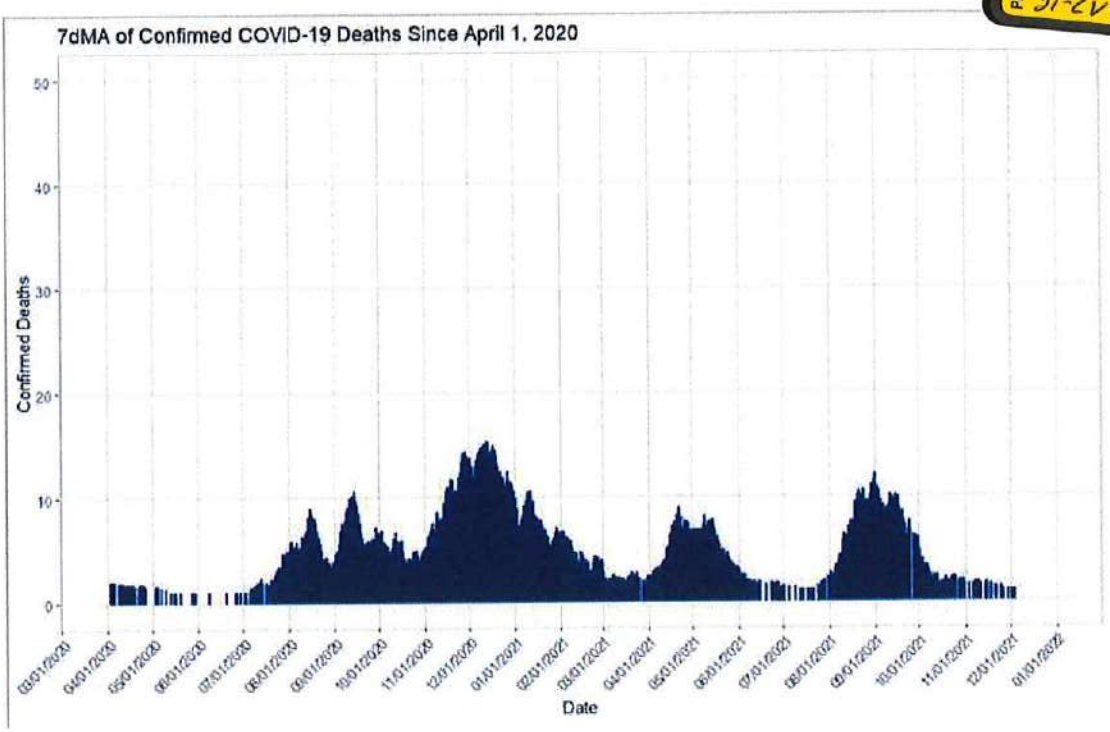
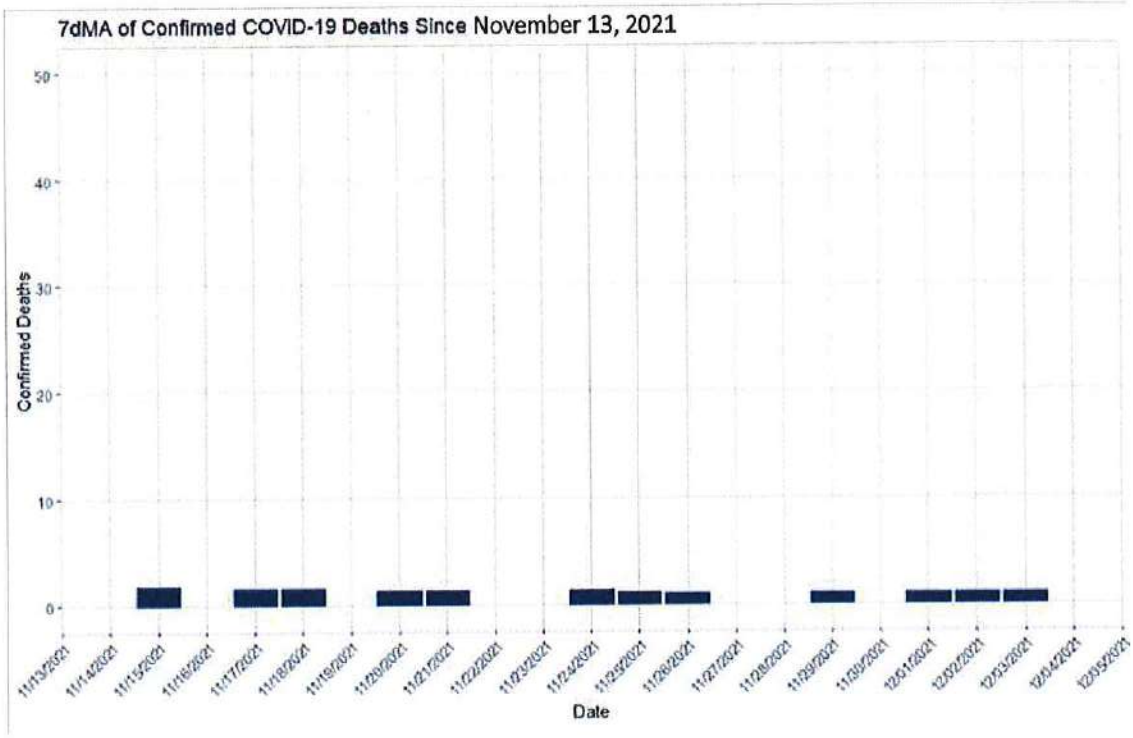


C, *New Hospital Admissions* (By Jurisdiction, Select Jurisdiction: Puerto Rico), https://covid.cdc.gov/covid-data-tracker/#new-hospital-admissions





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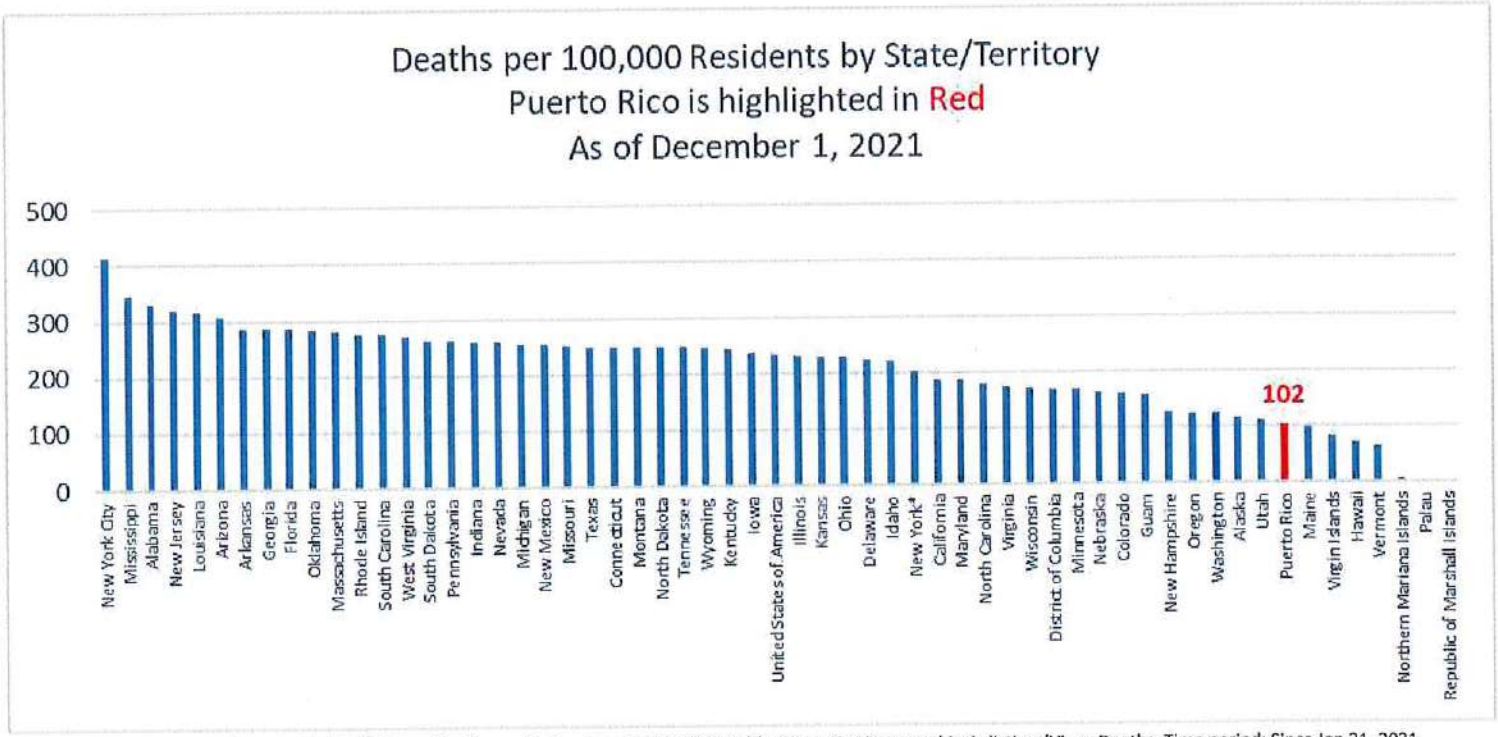
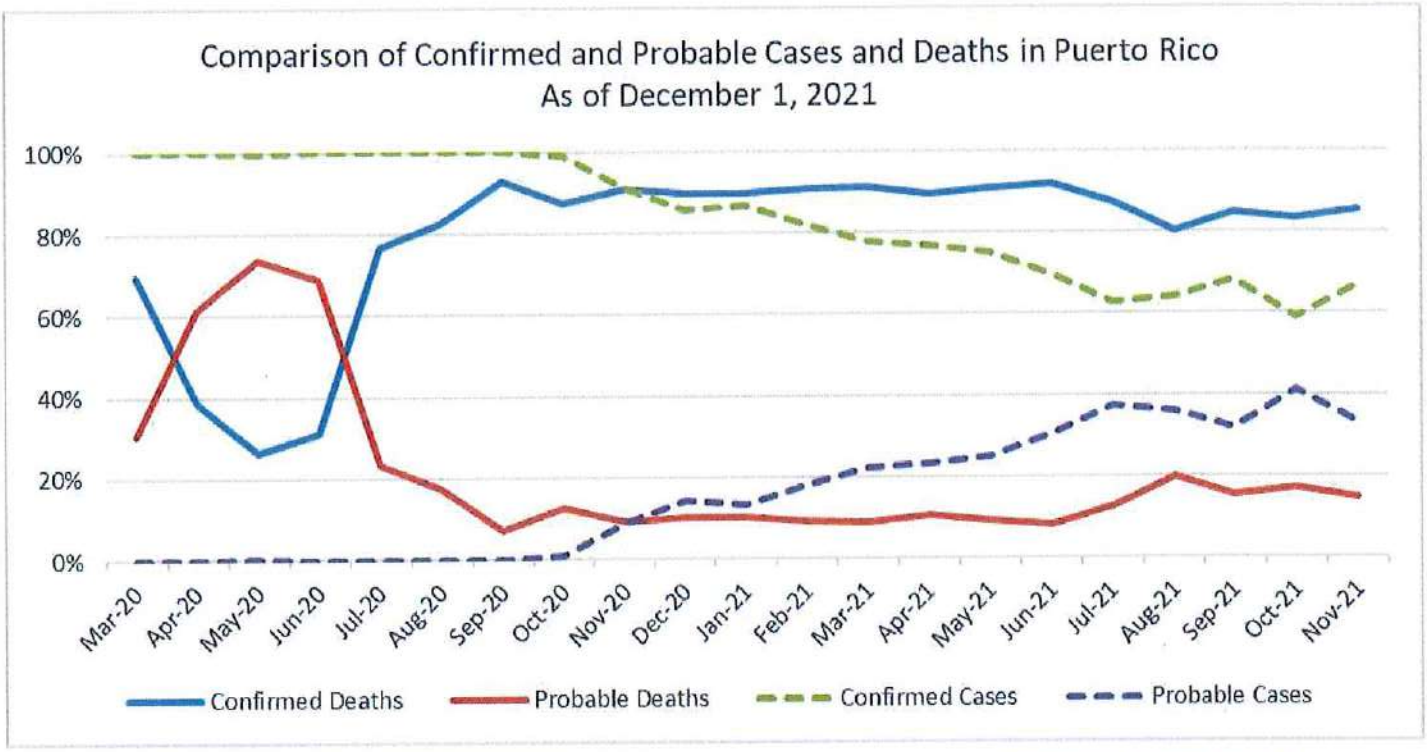
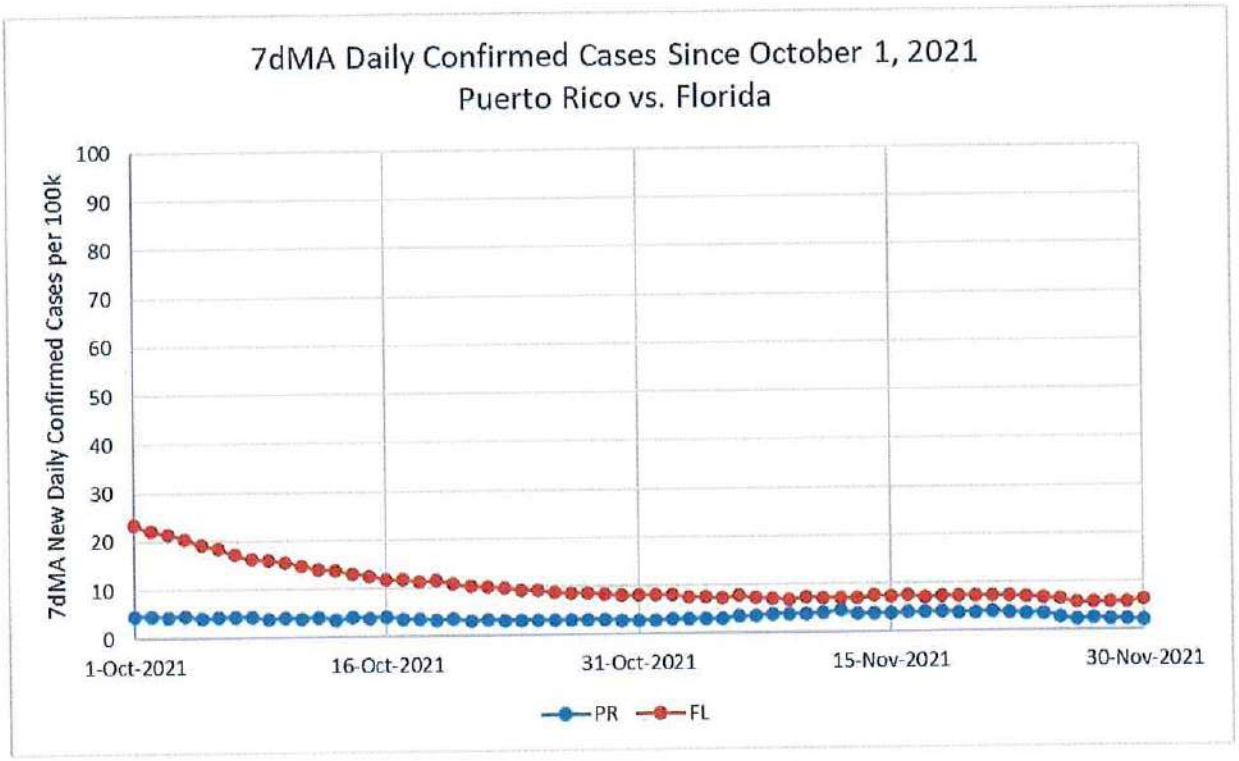


Data Source: CDC, United States COVID-19 Cases, Deaths, and Laboratory Testing (NAATs) by State, Territory, and Jurisdiction (View: Deaths, Time period: Since Jan 21, 2021, Metric: Rate per 100,000), *Data Table for Death Rate by State/Territory*, https://covid.cdc.gov/covid-data-tracker/#cases_deathsper100k



Data Sources: Puerto Rico Health Department. COVID-19 Dashboard, *Datos (Casos) and Datos (Defunciones)*, https://covid19datos.salud.gov.pr/



Data Sources:

- Puerto Rico Health Department. COVID-19 Dashboard, Datos (Casos), https://covid19datos.salud.gov.pr/
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Effectiveness and durability of protection against future SARS-CoV-2 infection conferred by COVID-19 vaccination and previous infection; findings from the UK SIREN prospective cohort study of healthcare workers March 2020 to September 2021

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ABSTRACT

Background:

Understanding the duration and effectiveness of infection and vaccine-acquired SARS-CoV-2 immunity is essential to inform pandemic policy interventions, including the timing of vaccine-boosters. We investigated this in our large prospective cohort of UK healthcare workers undergoing routine asymptomatic PCR testing.

Methods

We assessed vaccine effectiveness (VE) (up to 10-months after first dose) and infection-acquired immunity by comparing time to PCR-confirmed infection in vaccinated and unvaccinated individuals using a Cox regression-model, adjusted by prior SARS-CoV-2 infection status, vaccine-manufacturer/dosing-interval, demographics and workplace exposures.

Results

Of 35,768 participants, 27% (n=9,488) had a prior SARS-CoV-2 infection. Vaccine coverage was high: 97% had two-doses (79% BNT162b2 long-interval, 8% BNT162b2 short-interval, 8% ChAdOx1). There were 2,747 primary infections and 210 reinfections between 07/12/2020 and 21/09/2021. Adjusted VE (aVE) decreased from 81% (95% CI 68%-89%) 14-73 days after dose-2 to 46% (95% CI 22%-63%) >6-months; with no significant difference for short-interval BNT162b2 but significantly lower aVE (50% (95% CI 18%-70%) 14-73 days after dose-2 from ChAdOx1. Protection from infection-acquired immunity showed evidence of waning in unvaccinated follow-up but remained consistently over 90% in those who received two doses of vaccine, even in those infected over 15-months ago.

Conclusion

Two doses of BNT162b2 vaccination induce high short-term protection to SARS-CoV-2 infection, which wanes significantly after six months. Infection-acquired immunity boosted

63 with vaccination remains high over a year after infection. Boosters will be essential to
64 maintain protection in vaccinees who have not had primary infection to reduce infection and
65 transmission in this population.

66 Trial registration number

67 ISRCTN11041050

68

BACKGROUND

Understanding the durability of the immune response to SARS-CoV-2 infection and COVID-19 vaccination remains critical to the global COVID-19 response. Twenty months after emergence, SARS-CoV-2 has caused millions of deaths,[1] and widespread disruption to global health and economies. The development and mass deployment of COVID-19 vaccines within a year was unprecedented and has facilitated relaxation of non-pharmaceutical interventions. COVID-19 vaccines have demonstrated short-term effectiveness in real-world studies, reducing both symptomatic and asymptomatic infection, severity and secondary transmission.[2-5] The duration of this protection over longer periods remains uncertain and requires ongoing study.

Population uptake of COVID-19 vaccination in the UK (aged over 12 years) is 80.4% for two doses,[6] and prioritised groups (health and social care workers and the clinically vulnerable), are now over six months after their second dose. Following concerns about potential vaccine waning at this point,[7-11] and in the context of sustained high levels of community infections,[6] the UK Government initiated a roll-out of booster vaccination to priority groups in September 2021.[12] Improved understanding and characterisation of vaccine effectiveness at longer intervals and potential variation by demographic factors, vaccine schedules and history of SARS-CoV-2 infection is urgently required to support global vaccination schedules.

The SARS-CoV-2 Immunity and Reinfection Evaluation (SIREN) study, a large cohort of healthcare workers undergoing fortnightly asymptomatic PCR testing, had over 30% of participants testing seropositive at enrolment and is well suited to this task.[5,13,14] In this analysis we estimate the effectiveness and durability of protection against future SARS-CoV-2 infection conferred by previous SARS-CoV-2 infection and COVID-19 vaccination in the SIREN cohort from March 2020 to September 2021.

METHODS

Study design and participants

The SIREN study is a multicentre prospective cohort of healthcare workers aged over 18 years across the UK.

Data sources and measurement

Participants undergo fortnightly SARS-CoV-2 PCR testing (supplemented by widespread lateral flow testing), monthly antibody testing and complete regular questionnaires. This data collection is described elsewhere.[5]

Vaccination data (manufacturer, dates) were obtained via linkage on personal identifiers from national COVID-19 registries in each health administration and directly from participants in their questionnaires. Dosing interval was categorised as 'short' if dose-two was administered up to 6-weeks post dose-one and 'long' if ≥6-weeks.[15]

Serum samples from all participant baseline visits are collected centrally and tested at the United Kingdom Health Security Agency (UKHSA) central testing laboratory at Porton Down using the semi-quantitative Elecsys Anti-SARS-CoV-2 nucleocapsid (N) protein assay and fully quantitative Elecsys Anti-SARS-CoV-2 spike (S) protein assay (Roche Diagnostics).

Outcomes

The primary outcome was a PCR-confirmed SARS-CoV-2 infection, irrespective of symptom status, that met the definition of a reinfection in the positive cohort of two PCR positives ≥90 days or one new PCR positive ≥28 days after an antibody positive result consistent with previous infection.

Explanatory variables and exclusion criteria

Participants were assigned into one of two cohorts at the start of analysis time: participants in the naïve cohort had no history of SARS-CoV-2 positivity and the positive cohort being those who had ever received a PCR positive or antibody result consistent with prior SARS-CoV-2 infection.

Participants were excluded from this analysis if event or cohort assignment could not be accurately completed, i.e. no PCR tests during follow-up, or if they were in the positive cohort but were infected after vaccination or lacked an onset date for primary infection (PCR positive or COVID-symptom onset).

Person time at risk

Follow-up began on 07 December 2020, the day before COVID-19 vaccination was introduced to the UK, and continued until 21 September 2021, covering 10 calendar months. All participants enrolled on or before 07 December 2020 contributed follow-up time from 07 December 2020 onwards. Participants enrolled after 07 December 2020 began contributing follow-up time from their enrolment date (delayed entry). Participants moved from the negative to positive cohort 90 days after a primary PCR positive date, if their primary infection was before vaccination, at which point they were considered at risk of reinfection (mirroring the SIREN reinfection definition: two PCR positives >90-days apart). End of follow-up time for individual participants was either date of primary infection (negative cohort), date of reinfection (positive cohort) or last PCR negative test.

Statistical methods

We used a Cox proportional hazards model with delayed entry, the outcome being time-to-infection with a positive PCR test. The model accounted for the calendar time, varying infection rate via the baseline hazard, that could take any functional form. Analysis time started shortly before the second wave peaked, continuing through Spring 2021 and into the third wave (Supplementary Figure iii), thus, accounting for a varying hazard rate was crucial.

The main predictors – vaccine status and previous infection status - were categorical and time-varying. We grouped on the time to vaccination and divided follow-up time into unvaccinated and 24 post-vaccination time intervals, with post-vaccination intervals categorised by manufacturer, dose and dosing interval, the latter to explore differences in protection in those receiving dose two closer in time to their first dose. We also grouped the

time since primary infection into four time-intervals: before primary infection (naïve), and then 3-9 months, 9-15 months and ≥15 months after primary infection. Vaccine effectiveness and protection from primary infection were calculated as 1-HR. We used robust variance estimates to guard against the potential for unmeasured confounders at trust level.

We initially fitted a main effects model, with no interactions between vaccine and primary infection status. This was our main model that highlighted vaccine effectiveness over time. We also fitted an interaction model, in which we did not consider time since vaccination, brand and manufacturer, to focus on protection from primary infection over time by vaccine status. We fitted both models with and without additional time invariant covariates: age, ethnicity, co-morbidities, region, frequency of COVID-19 patient contact, patient-facing role, and workplace setting. Independently, we also fitted an equivalent piecewise exponential proportional hazards model. This produced consistent VE results and provided estimates of the baseline hazard rates (supplementary material; Figure iii), analogous to the method we have previously described.[5] We used STATA software (version 15.1; StataCorp LLC, College Station, TX, USA) for all analyses. Results were independently replicated in R (v. 4.1.1, survival package v.3.2-13). Our annotated code is available (https://github.com/SIREN-study/SARS-CoV-2-Immunity).

This study was registered, number ISRCTN11041050, and received approval from the Berkshire Research Ethics Committee on 22 May 2020. Reporting of the study follows the Strengthening the Reporting of Observational studies in Epidemiology guidelines.[16]

RESULTS

Study population

The SIREN study enrolled 44,546 participants between 18 June 2020 and 23 April 2021 from 135 sites across the UK; n=35,768 met the inclusion criteria for this analysis (Supplementary Figure i). Participants are described in Table 1, and were predominantly

172 female (84%), with a median age of 46 years (IQR 36-54). We assigned 26,280 participants
173 to the naïve cohort and 9,488 to the positive cohort at analysis start time. The positive
174 cohort were more likely to be male, younger, from Black, Asian and ethnic minority
175 backgrounds, work in clinical roles and report more frequent exposure to COVID-19 patients
176 (Table 1). By the end of analysis time, 97% of the cohort had received two vaccine doses:
177 78.5% BNT162b2 long-interval, 8.6% BNT162b2 short-interval and 7.8% ChAdOX1 (Table
178 1, Supplementary Figure ii). We identified no major demographic differences between
179 participants by vaccine schedule (Supplementary Table i). Follow-up time varied by
180 participant, with a total of 7,482,388 participant person-days, of which there were 998,270
181 person-days unvaccinated, and 6,430,118 person-days vaccinated (from date of first dose).
182 There were 60,301 PCR tests performed in the unvaccinated follow-up period and 443,979
183 PCR tests in the vaccinated follow-up period, with an average test interval of 16.6 days per
184 test in the unvaccinated period and 14.5 days per test in the vaccinated period. There were
185 2,747 primary infections during follow-up and 210 reinfections, with cases peaking at the end
186 of December 2020 and declining by March-April 2021, before increasing in May 2021, which
187 mirrored national trends (Supplementary Figure iii).

188 **Vaccine effectiveness**

189 The overall adjusted vaccine effectiveness (aVE) against infection following dose-two of
190 BNT162b2 vaccine administered after the long-interval was 81% (95% CI 68%-89%) in the
191 first two months after the development of the full immune response (14-73 days after second
192 dose) (Table 2, Figure 1). aVE declined over time, although remained high at 70% (95% CI
193 62%-76%) 4-6 months after dose-two. After six months we saw evidence of waning, with
194 aVE of 46% (95% CI 22%-63%).

195 A similar trend was observed for BNT162b2 dose two short-interval, with higher protection at
196 14-73 days (aVE 86% (95% CI 73%-93%) decreasing to 61% (95% CI 45%-73%) after 6-
197 months. We found no significant difference in protection after dose-two between BNT162b2

198 long and short inter-vaccination intervals, with HR for infection of 1.39 (95% CI 0.64-3.00,
199 p=0.41) using short interval as the reference group.

200 For ChAdOX1, aVE from two doses was 50% (95% CI 16%-69%) 14-73 days after second
201 dose. Effectiveness did not fall significantly after longer intervals after dose-two, with
202 overlapping confidence intervals of VE reflecting the small number of participants
203 contributing to this estimate (Table 2, Figure 1). Compared to ChAdOX1, we found that
204 Pfizer short was 72% more effective (95% CI 38%-88%, p=0.002) and Pfizer long was 62%
205 more effective (95% CI 26%-80%, p=0.004), in the interval 14-73 days.

206 **Durability of protection following primary infection**

207 In contrast, looking at the impact of vaccination on the cohort with prior COVID-19 infection
208 (positive cohort), using naïve unvaccinated as the reference group (Table 3, Figure 2), a
209 beneficial boosting of infection-acquired immunity was apparent, with combined protection
210 over 90% a year or more after primary infection and two doses of vaccination. There was no
211 evidence of the protection afforded by primary infection waning in participants who had
212 received two doses of vaccine up to 15 months after the primary infection. A similar trend
213 was observed after a single dose and even without vaccination, however, most unvaccinated
214 follow-up time occurred pre-Delta.

215 **DISCUSSION**

216 Eighteen months after the emergence of SARS-CoV-2 and ten months after the rapid
217 deployment of COVID-19 vaccines, we have assessed the durability of protection from
218 SARS-CoV-2 infection conferred by both infection-acquired and vaccine-acquired immunity.
219 Our cohort of 35,768 healthcare workers, including over a quarter with prior infection,
220 primarily received two doses of BNT162b2 administered at a long inter-vaccine interval,
221 which induced high levels of protection over the first 6 months, peaking between 68% and
222 89% in the first two months; however, we found evidence of significant waning, with
223 protection reducing to between 22% and 63% after six months. We found no difference in

protection following two doses when comparing BNT162b2 short interval with BNT162b2 long interval, although we found significantly lower protection from two doses of ChAdOX1 compared to BNT12b2. Of note, the period of waning coincided with the Delta variant being the predominant circulating strain, which may account for the more pronounced waning of protection in our cohort, given the reduced vaccine effectiveness against Delta reported.[17] Delivery of vaccination to individuals after prior infection effectively boosts and extends their immunity, with participants who received two doses of vaccination after infection emerging as the most highly protected group in our cohort for both symptomatic and asymptomatic infection, with similar protection to that provided by a three-course vaccination against symptomatic infection.[18]

Our finding of reduced protection from infection following two doses of vaccination after six months strengthens the accruing evidence base. Our design overcomes several biases of recent studies, including underestimation of the proportion with prior infection;[19] previous studies have typically investigated symptomatic infection and utilised test-negative case-control or retrospective cohort designs using national testing surveillance data.[7,9,11] We note that these real-world studies have found consistently lower protection and more pronounced waning than the recent BNT162b2 clinical trial, which reported vaccine efficacy against symptomatic infection of 83.7% (95% CI, 74.7 to 89.9) 4-6 months after dose-2,[20] likely related to the reduced vaccine effectiveness reported against the Delta variant.[17] The significantly lower protection observed in this study after ChAdOX1 compared to BNT162b2 has also been found in other recent studies.[7,20] Several studies have observed lower antibody titres following ChAdOx1 vaccination than BNT162b2,[21,22] and a shorter interval to fall below a protective antibody threshold from this lower baseline has been proposed as a causal mechanism for the lower vaccine effectiveness.[20] We found no evidence of a difference in protection against infection after two doses of BNT162b2 between short and long-interval. This is despite evidence of significantly higher antibody, B cell and T cell responses in recipients of long-interval compared to short-interval vaccination

regimens,[15,23,24] and higher VE against symptomatic infection from one observational study.[15] Plausibly the threshold to prevent all infections may be lower than that for symptomatic infection.

Studies to date have shown more durable protection against severe outcomes of hospitalisation and death following vaccination.[7,25] Whilst we have estimated VE against all infections, including asymptomatic infections that have limited clinical impact, a reduction in VE against infection will increase transmission and risk of infection to high- risk individuals, some of whom will progress to severe disease.

To our knowledge, this study reports the longest real-world follow-up time from primary infection to date. It remains unclear how long immune protection will last after previous infection due to the limited length of follow-up period, however modelling has suggested that protection could last for up to 61 months.[21,26] In our cohort, we have demonstrated that protection from primary infection can last up to 15 months in some individuals, while other studies have reported protection ranging from 5-12 months.[27-29] Our ability to study infection-acquired immunity in unvaccinated individuals at longer intervals is now limited given the very small number of our cohort remaining unvaccinated. It is important to highlight that most follow-up without vaccination in the 9-15-month category occurred in the pre-Delta wave. It is possible that the sustained infection-acquired protection in our cohort is affected by repeated low dose occupational exposure to COVID-19,[30] and therefore less generalisable to populations at lower exposure. It is also possible that this results from a broader diversity of T-cell immunity against different SARS-CoV-2 spike protein epitopes emerging following infection, enhancing protection against variants and inducing long-lasting memory T-cell populations.[27,31,32] Although our finding of greater protection following infection-acquired immunity has been demonstrated by other authors,[33,34] others have reported vaccine-acquired immunity to be equivalent,[35,36] or superior.[37] Despite the high protection provided by infection-acquired immunity, we have demonstrated additional benefit from vaccination in previously infected participants, in line with previous studies.[34,38,39] Until

thresholds for protective antibody titres against SARS-CoV-2 infection are established, it is challenging to accurately estimate how much vaccine-induced immunity is required to prevent reinfection at an individual level.

Key strengths of our study are the size of our cohort, asymptomatic testing and testing frequency, with an average PCR test interval of 16.6 days in unvaccinated time and 14.5 days per test in vaccinated follow-up time, supplemented by the widespread use of lateral flow testing, which means we can be confident that most infections were detected. As a well-defined cohort, we can simultaneously investigate vaccination and prior infection status and adjust for important confounders, including workplace exposures. The most important limitation of our study is the relatively small number of participants continuing to contribute follow-up time to key vaccination exposures: unvaccinated, ChAdOx1 and BNT162b2 short interval. This particularly affects the precision of estimates and our ability to assess potential waning following two-doses of ChAdOx1, and >15 months after primary infection in unvaccinated participants. We consider that the strengths of our study design and speed of vaccine deployment significantly limit the impact of depletion-of-susceptible bias (which particularly affects studies on vaccine-waning),[19] however we recognise some residual confounding may remain.

Conclusion

Two doses of BNT162b2 vaccination, given with a short or long-interval, induce high protection to SARS-CoV-2 infection (asymptomatic and symptomatic) in the short-term, but this protection wanes after six months, during a period where Delta predominates. Protection provided from two doses of ChAdOX1 is considerably lower overall. The highest and most durable protection is observed in those with hybrid immunity, who received one or two doses of vaccine after a primary infection; this will be important for the deployment of vaccines in highly exposed and immune populations. Strategic use of booster vaccine doses to avert waning of protection (particularly in double vaccinated naïve individuals) is

304 essential to provide reduced infection, and therefore transmission in the ongoing global
305 response to COVID-19.

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310

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Tables and Figures

Table 1: Description of participant demographics, by cohort assignment, June 2020 to September 2021

Demographics	Total n (%)	Naïve cohort n (%)	Positive cohort n (%)	p-value
Gender				
Male	5699 (15.9)	4051 (15.4)	1648 (17.4)	<0.0001
Female	30017 (83.9)	22190 (84.4)	7827 (82.5)	<0.0001
Other	52 (0.1)	39 (0.1)	13 (0.1)	-
Age group				
Under 25	1297 (3.6)	935 (3.6)	362 (3.8)	0.3734
25 to 34	7106 (19.9)	5023 (19.1)	2083 (22.0)	<0.0001
35 to 44	8848 (24.7)	6580 (25.0)	2268 (23.9)	0.0332
45 to 54	10874 (30.4)	8007 (30.5)	2867 (30.2)	0.5861
55 to 64	7085 (19.8)	5283 (20.1)	1802 (19.0)	0.0212
Over 65	558 (1.6)	452 (1.7)	106 (1.1)	<0.0001
Ethnicity				
White	31634 (88.4)	23610 (89.8)	8024 (84.6)	<0.0001
Asian	2486 (7.0)	1581 (6.0)	905 (9.5)	<0.0001
Black	621 (1.7)	381 (1.4)	240 (2.5)	<0.0001
Mixed race	535 (1.5)	380 (1.4)	155 (1.6)	0.1629
Other ethnic group	427 (1.2)	278 (1.1)	149 (1.6)	0.0002
Prefer not to say	65 (0.2)	50 (0.2)	15 (0.2)	-
Medical conditions category				
No medical condition	26670 (74.6)	19569 (74.5)	7101 (74.8)	0.5651
Immunosuppression	803 (2.2)	623 (2.4)	180 (1.9)	0.005
Chronic respiratory conditions	4439 (12.4)	3306 (12.6)	1133 (11.9)	0.0763
Chronic non-respiratory conditions	3856 (10.8)	2782 (10.6)	1074 (11.3)	0.0596
Staff group				
Administrative/Executive (office based)	5434 (15.2)	4280 (16.3)	1154 (12.2)	<0.0001
Nursing	12184 (34.1)	8658 (32.9)	3526 (37.2)	<0.0001
Healthcare Assistant	2901 (8.1)	1994 (7.6)	907 (9.6)	<0.0001
Doctor	4248 (11.9)	3053 (11.6)	1195 (12.6)	0.0098
Midwife	777 (2.2)	582 (2.2)	195 (2.1)	0.5669
Physiotherapist/Occupational Therapist/SALT	1438 (4.0)	996 (3.8)	442 (4.7)	0.0001
Estates/Porters/Security	530 (1.5)	389 (1.5)	141 (1.5)	-
Pharmacist	737 (2.1)	582 (2.2)	155 (1.6)	0.0004
Healthcare Scientist	1390 (3.9)	1147 (4.4)	243 (2.6)	<0.0001
Student (Medical/Nursing/Midwifery/Other)	1200 (3.4)	867 (3.3)	333 (3.5)	0.3536
Other	4929 (13.8)	3732 (14.2)	1197 (12.6)	0.0001
Occupational setting				
Office based	7002 (19.6)	5481 (20.9)	1521 (16.0)	<0.0001
Patient facing (non-clinical)	1378 (3.9)	1064 (4.0)	314 (3.3)	0.0023
Outpatient	7341 (20.5)	5662 (21.5)	1679 (17.7)	<0.0001

Maternity/Labour Ward	477 (1.3)	361 (1.4)	116 (1.2)	0.1475
Ambulance/Emergency Department/Inpatient Wards	6456 (18.0)	4225 (16.1)	2231 (23.5)	<0.0001
Intensive Care	1669 (4.7)	1273 (4.8)	396 (4.2)	0.0173
Theatres	866 (2.4)	657 (2.5)	209 (2.2)	0.1031
Other	10579 (29.6)	7557 (28.8)	3022 (31.9)	<0.0001
Patient contact				
No	5105 (14.3)	4053 (15.4)	1052 (11.1)	<0.0001
Yes	30663 (85.7)	22227 (84.6)	8436 (88.9)	<0.0001
Frequency of COVID-19 patient contact				
Never	12752 (35.7)	10290 (39.2)	2462 (25.9)	<0.0001
Every day	8797 (24.6)	5585 (21.3)	3212 (33.9)	<0.0001
Once week	6229 (17.4)	4340 (16.5)	1889 (19.9)	<0.0001
Once month	3257 (9.1)	2368 (9.0)	889 (9.4)	0.2457
Less month	4733 (13.2)	3697 (14.1)	1036 (10.9)	<0.0001
Index of Multiple Deprivation				
5 (least deprived)	8871 (24.8)	6563 (25.0)	2308 (24.3)	0.176
4	8073 (22.6)	5982 (22.8)	2091 (22.0)	0.1102
3	7515 (21.0)	5537 (21.1)	1978 (20.8)	0.5387
2	6020 (16.8)	4408 (16.8)	1612 (17.0)	0.6555
1 (most deprived)	3858 (10.8)	2680 (10.2)	1178 (12.4)	<0.0001
Not known	1431 (4.0)	1110 (4.2)	321 (3.4)	0.0006
Geographical area				
East Midlands	2825 (7.9)	1963 (7.5)	862 (9.1)	<0.0001
East of England	3363 (9.4)	2415 (9.2)	948 (10.0)	0.0222
London	3688 (10.3)	2432 (9.3)	1256 (13.2)	<0.0001
North East	647 (1.8)	453 (1.7)	194 (2.0)	0.0582
North West	3429 (9.6)	2174 (8.3)	1255 (13.2)	<0.0001
South East	3548 (9.9)	2568 (9.8)	980 (10.3)	0.1628
South West	5540 (15.5)	4503 (17.1)	1037 (10.9)	<0.0001
West Midlands	2717 (7.6)	1900 (7.2)	817 (8.6)	<0.0001
Yorkshire and Humber	2644 (7.4)	1765 (6.7)	879 (9.3)	<0.0001
Scotland	5449 (15.2)	4646 (17.7)	803 (8.5)	<0.0001
Northern Ireland	1127 (3.2)	888 (3.4)	239 (2.5)	<0.0001
Wales	791 (2.2)	573 (2.2)	218 (2.3)	0.5715
Vaccination status by 21 Sep 21				
2-doses BNT162b2 Long interval	28078 (78.5)	21427 (79.2)	6651 (76.4)	<0.0001
2-doses BNT162b2 Short interval	3059 (8.6)	2493 (9.2)	566 (6.5)	<0.0001
2-doses ChAdOX1	2803 (7.8)	2002 (7.4)	801 (9.2)	<0.0001
1-dose (any)	937 (2.6)	652 (2.4)	285 (3.3)	<0.0001
Unvaccinated	891 (2.5)	483 (1.8)	408 (4.7)	<0.0001
Total	**35,768**	**26,280 (73.5)**	**9,488 (26.5)**	

Positive cohort assignment: 83% seropositive (72% on UKHSA testing), 17% seronegative with historic antibody/PCR positive). Primary infections in the positive cohort occurred in March-May 2020 for 2,576 (57.6%) participants, June-August for 167 (3.7%) and September-December for 1,728 (38.6%). * Index of Multiple Deprivation (IMD), which is a measure of neighbourhood relative deprivation calculated by the Office of National Statistics, was obtained through linkage with participant postcodes

Table 2: Incidence of SARS-CoV-2 infections and effectiveness of COVID-19 vaccines against infection by dose, manufacturer and dosing interval, SIREN participants 07 December 2020 to 21 September 2021

Vaccine status	Number of participants	Number of days of follow up	All infections (symptomatic & asymptomatic)			
			Number of infections	Crude Incident rate (per 10,000)	VE (1-HR) 95% CI	aVE (1-HR) 95% CI
Unvaccinated	24,787	995,122	1067	10.72	Reference	Reference
Vaccinated 1 dose						
Time since vaccine						
BNT162b2						
21 – 27 days	21,374	141,696	55	3.88	0.56 (0.40-0.68)	0.57 (0.41-0.69)
28 – 41 days	21,137	279,672	62	2.22	0.62 (0.46-0.74)	0.62 (0.46-0.74)
42 – 55 days	21,968	289,896	32	1.10	0.68 (0.52-0.78)	0.67 (0.51-0.78)
> 55 days	23,049	477,746	60	1.26	0.64 (0.51-0.73)	0.58 (0.42-0.70)
ChAdOX1						
21 – 27 days	2,299	15,848	3	1.89	0.46 (-0.82-0.84)	0.42 (-0.92-0.83)
28 – 41 days	2,423	32,556	1	0.31	0.88 (0.17-0.98)	0.87 (0.10-0.98)
42 – 55 days	2,488	33,514	3	0.90	0.46 (-0.56-0.81)	0.40 (-0.75-0.80)
> 55 days	2,503	64,708	10	1.55	0.36 (-0.30-0.68)	0.29 (-0.43-0.65)
Vaccinated 2 doses						
Time since vaccine						
BNT162b2 long-interval						
14 – 73 days	25,571	1,466,353	26	0.18	0.83 (0.70-0.90)	0.81 (0.68-0.89)
74 – 133 days	23,776	1,297,486	285	2.20	0.70 (0.61-0.77)	0.65 (0.56-0.73)
134 – 193 days	18,255	688,494	505	7.33	0.70 (0.61-0.77)	0.67 (0.58-0.75)
>193 days	2,704	24,575	83	33.77	0.43 (0.16-0.61)	0.43 (0.17-0.61)
BNT162b2 short-interval						
14 – 73 days	2,861	151,318	10	0.66	0.85 (0.70-0.92)	0.85 (0.71-0.92)
74 – 133 days	2,822	164,199	6	0.37	0.72 (0.40-0.87)	0.72 (0.42-0.86)
134 – 193 days	2,659	147,301	50	3.39	0.56 (0.38-0.69)	0.55 (0.37-0.67)
>193 days	2,105	84,705	90	10.63	0.59 (0.41-0.71)	0.58 (0.40-0.71)
ChAdOX1						
14 – 73 days	2,394	133,865	19	1.42	0.52 (0.20-0.71)	0.49 (0.16-0.69)
74 – 133 days	2,003	92,621	55	5.94	0.53 (0.34-0.67)	0.47 (0.26-0.63)
> 133 days	995	23,226	32	13.78	0.59 (0.31-0.75)	0.51 (0.18-0.71)

Number of infections includes both primary infections and reinfections (all PCR confirmed)

Crude incident rate: number of infections/days of follow-up (*10,000), does not adjust for variable baseline hazard.

VE: unadjusted Vaccine Effectiveness, model adjusted for time since vaccination and previous infection status (time since previous infection).

aVE: adjusted Vaccine Effectiveness, model adjusted for time since vaccination and previous infection status (time since previous infection) and constant predictors: age, gender, ethnicity, comorbidities, workplace setting, frequency of contact with COVID-19 patients, geographical area (of workplace). More details are available in supplementary Table i

Table 3: Incidence of SARS-CoV-2 reinfections and durability of protection against SARS-CoV-2 reinfection, adjusted for vaccine status, in the SIREN cohort between 07 December 2020 and 21 September 2021

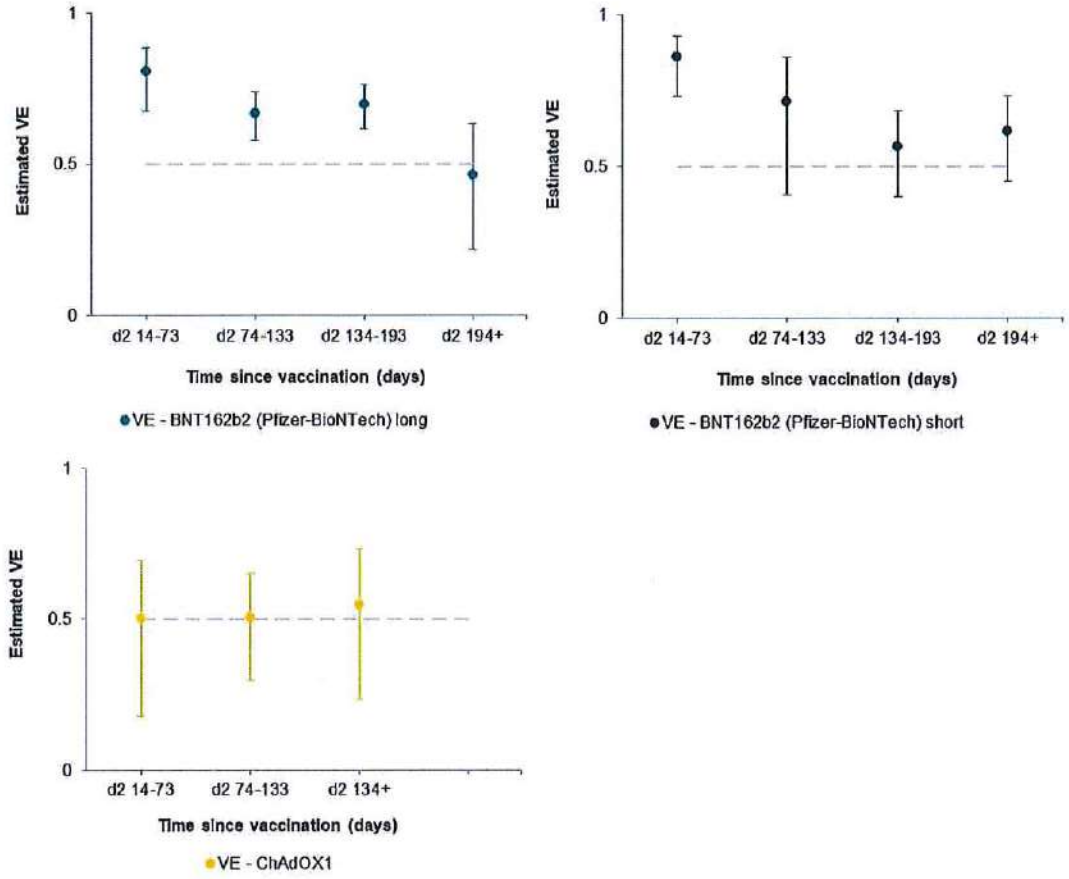
Vaccine and prior infection status	Number of participants	Number of days of follow up	Number of infections	Crude incident rate (per 10,000)	Adjusted Absolute protection against infection (1-HR) 95% CI
Unvaccinated					
Naïve	16,039	646,495	983	15.21	Reference
Prior infection 3-9 months	6,173	169,697	41	2.42	0.85 (0.80-0.89)
Prior infection 9-15 months	4,145	163,437	32	1.96	0.85 (0.78-0.89)
Prior infection ≥15 months	291	15,493	11	7.10	0.73 (0.43-0.87)
Vaccinated dose 1					
Naïve	21,263	1,273,056	607	4.77	0.35 (0.24-0.44)
Prior infection 3-9 months	4,561	152,160	15	0.99	0.87 (0.79-0.92)
Prior infection 9-15 months	5,978	358,618	24	0.67	0.90 (0.86-0.93)
Prior infection ≥15 months	196	7,353	2	2.72	0.87 (0.50-0.97)
Vaccinated dose 2					
Naïve	22,586	3,414,257	1102	3.23	0.64 (0.56-0.70)
Prior infection 3-9 months	2,928	320,252	24	0.75	0.91 (0.86-0.94)
Prior infection 9-15 months	7,202	624,026	29	0.46	0.91 (0.86-0.94)
Prior infection ≥15 months	4,980	280,388	30	1.07	0.95 (0.93-0.97)

Number of infections includes both primary infections and reinfections (all PCR confirmed)

Crude incident rate: number of infections/days of follow-up (*10,000), does not adjust for variable baseline hazard.

Adjusted absolute protection against infection: model adjusted for previous infection status (time since previous infection), vaccine status (unvaccinated, dose 1 (this includes follow-up between dose 1 and dose 2), dose 2 (includes follow-up after dose 2)) and constant predictors: age, gender, ethnicity, comorbidities, workplace setting, frequency of contact with COVID-19 patients, geographical area (of workplace). Reference group is unvaccinated infection naïve. The model does not adjust for time since vaccination (as in table 2), vaccine manufacturer or vaccine dosing interval, therefore please refer to Table 2 for any vaccine effectiveness estimates.

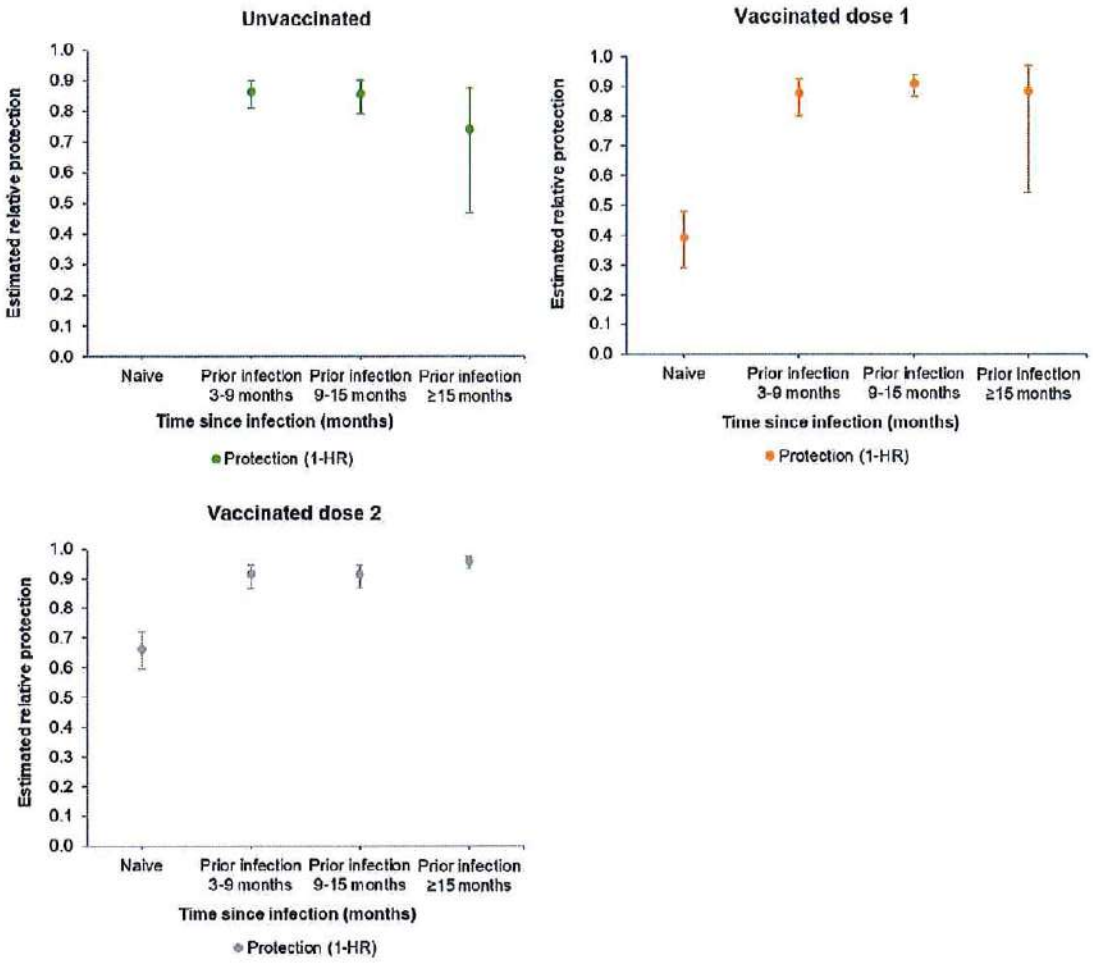
Figure 1: Adjusted Vaccine Effectiveness over time after two doses: BNT162b2 (Pfizer-BioNTech) short and long interval and ChAdOX1 (combined short and long interval)



Number of participants: **BNT162b2 long-interval:** 14-73 days n=25571, 74-133 days n=23776, 134-193 days n=18255, over 193 days n=2704; **BNT162b2 short-interval**: 14-73 days n=2861, 74-133 days n=2822, 134-193 days n=2659, over 193 days n=2105; **ChAdOx1**: 14-73 days n=2394, 74-133 days n=2003, over 133 days n=995.

aVE: adjusted Vaccine Effectiveness, model adjusted for time since vaccination and previous infection status (time since previous infection) and constant predictors: age, gender, ethnicity, comorbidities, workplace setting, frequency of contact with COVID-19 patients, geographical area (of workplace).

Figure 2: Protection following primary infection under different COVID-19 vaccination scenarios, up to 18 months following infection



Number of participants: **Unvaccinated:** prior infection 3-9 months n=6298, 9-15 months n=4147 and over 15 months n=291; **vaccinated dose 1**: naïve n=21283, prior infection 3-9 months n=4561, 9-15 months n=5978 and over 15 months n=196; **vaccinated dose 2**: naïve n=22586, prior infection 3-9 months n=2928, 9-15 months n=7202 and over 15 months n=4980.

Adjusted absolute protection against infection: model adjusted for previous infection status (time since previous infection), vaccine status (unvaccinated, dose 1 (this includes follow-up between dose 1 and dose 2), dose 2 (includes follow-up after dose 2)) and constant predictors: age, gender, ethnicity, comorbidities, workplace setting, frequency of contact with COVID-19 patients, geographical area (of workplace). Reference group is unvaccinated infection naïve. The model does not adjust for time since vaccination (as in table 2), vaccine manufacturer or vaccine dosing interval.

□The SIREN study group

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35	Golden Jubilee National Hospital	Catherine	Sinclair
		Val	Irvine
36	GREAT WESTERN HOSPITALS NHS FOUNDATION TRUST	Eva	Fraile
37	HAMPSHIRE HOSPITALS NHS FOUNDATION TRUST	Claire	Thomas
		Ina	Hoad
38	HOUNSLOW AND RICHMOND COMMUNITY HEALTHCARE NHS TRUST	Shekoo	Mackay
		Shivani	Khan
39	HULL UNIVERSITY TEACHING HOSPITALS NHS TRUST	Philippa	Burns
		Nicholas	Easom
40	Hywel Dda University LHB	Tracy	Lewis
		Zohra	Omar
41	IMPERIAL COLLEGE HEALTHCARE NHS TRUST	Graham	Pickard
		Kenisha	Lewis
42	ISLE OF WIGHT NHS TRUST	Sarah	Hinch
		Alison	Brown
43	JAMES PAGET UNIVERSITY HOSPITALS NHS FOUNDATION TRUST	Ben	Burton
		Christian	Hacon
44	KING'S COLLEGE HOSPITAL NHS FOUNDATION TRUST	Ray	Chaudhuri
		Jonnie	Aeron-Thomas
45	LANCASHIRE & SOUTH CUMBRIA NHS FOUNDATION TRUST	Robert	Shorten
		Kathryn	Williams
46	LANCASHIRE TEACHING HOSPITALS NHS FOUNDATION TRUST	Maya	Leach
		Robert	Shorten
47	LEEDS TEACHING HOSPITALS NHS TRUST	Kyra	Holliday
		Clair	Favager
48	LEICESTERSHIRE PARTNERSHIP NHS TRUST	Sarah	Baillon
		Samantha	Hamer
49	LEWISHAM AND GREENWICH NHS TRUST	A	Shah
		J	Russell
50	LINCOLNSHIRE PARTNERSHIP NHS FOUNDATION TRUST	Kelly	Moran
		Ananta	Dave
51	LIVERPOOL UNIVERSITY HOSPITALS NHS FOUNDATION TRUST	Anu	Chawla
		Fran	Westwell
52	LONDON NORTH WEST UNIVERSITY HEALTHCARE NHS TRUST	Ekaterina	Watson
		D	Adeboyeku
53	MAIDSTONE AND TUNBRIDGE WELLS NHS TRUST	C	Pegg
		M	Williams
54	MANCHESTER UNIVERSITY NHS FOUNDATION TRUST	S	Ahmad
		A	Horsley
55	MID CHESHIRE HOSPITALS NHS FOUNDATION TRUST	Claire	Gabriel
		Katherin	Pagett
56	MID ESSEX HOSPITAL SERVICES NHS TRUST	Lauren	Sach
		Yvonne	Lester

57	MID YORKSHIRE HOSPITALS NHS TRUST	Ismaelette	Del Rosario
		John	Ashcroft
58	MOORFIELDS EYE HOSPITAL NHS FOUNDATION TRUST	Roxanne	Crosby-Nwaobi
		Chloe	Reeks
59	NHS Borders	Joy	Dawson
		Lauren	Finlayson
60	NHS Fife	Susan	Fowler
		Devesh	Dhasmana
61	NHS Forth Valley	Euan	Cameron
		Anne	Todd
62	NHS Grampian	Harriet	Carroll
		Alison	Thornton
63	NHS Greater Glasgow and Clyde	Antonia	Ho
		Michael	Murphy
64	NHS Highland	Andrew	Gibson
		Alexandra	Cochrane
65	NHS Lanarkshire	Manish	Patel
		Karen	Black
66	NHS Lothian	Kate	Templeton
		Andrea	Clarke
67	NHS Western Isles	Martin	Malcolm
		Joan	Frieslick
68	NORFOLK AND NORWICH UNIVERSITY HOSPITALS NHS FOUNDATION TRUST	Ngozi	Elumogo
		Louise	Coke
69	NORTH CUMBRIA INTEGRATED CARE NHS FOUNDATION TRUST	Beverly	Wilkinson
		John	Elliott
70	NORTH MIDDLESEX UNIVERSITY HOSPITAL NHS TRUST	Mariyam	Mirfenderesky
		Pratap	Harbham
71	NORTH WEST ANGLIA NHS FOUNDATION TRUST	Janki	Bhayani
		Stephanie	Diaz
72	NORTHERN DEVON HEALTHCARE NHS TRUST	M	Howard
		T	Lewis
73	NORTHERN HEALTH AND SOCIAL CARE TRUST	Elinor	Hanna
		Frances	Johnston
74	NORTHERN LINCOLNSHIRE AND GOOLE NHS FOUNDATION TRUST	Jonathan	Hatton
		Peter	Cowling
75	NOTTINGHAM UNIVERSITY HOSPITALS NHS TRUST	Sarah	Brand
		Imogen	Gould
76	POOLE HOSPITAL NHS FOUNDATION TRUST	Beverley	Wadams
		Elizabeth	Sheridan
77	PORTSMOUTH HOSPITALS NHS TRUST	Johanna	Mouland
		Jade	Yates
78	Powys Teaching LHB	Jayne	Goodwin
		Chris	Norman

79	QUEEN VICTORIA HOSPITAL NHS FOUNDATION TRUST	J	Giles
		G	Pottinger
80	ROYAL BERKSHIRE NHS FOUNDATION TRUST	Maya	Joseph
		Holly	Coles
81	ROYAL CORNWALL HOSPITALS NHS TRUST	H	Chenoweth
		D	Browne
82	ROYAL DEVON AND EXETER NHS FOUNDATION TRUST	Cressida	Auckland
		Stephanie	Prince
83	ROYAL FREE LONDON NHS FOUNDATION TRUST	Alison	Rodger
		Tabitha	Mahungu
84	ROYAL NATIONAL ORTHOPAEDIC HOSPITAL NHS TRUST	Esther	Hanison
		Simon	Warren
85	ROYAL PAPWORTH HOSPITAL NHS FOUNDATION TRUST	Sumita	Pai
		Helen	Baxendale
86	ROYAL SURREY COUNTY HOSPITAL NHS FOUNDATION TRUST	Charles	Piercy
		Esther	Tarr
87	ROYAL UNITED HOSPITALS BATH NHS FOUNDATION TRUST	Debbie	Delgado
		Sarah	Meisner
88	SALISBURY NHS FOUNDATION TRUST	Catherine	Thompson
		Sophia	Strong-Sheldrake
89	SANDWELL AND WEST BIRMINGHAM HOSPITALS NHS TRUST	Ash	Turner
		Anne	Hayes
90	SHEFFIELD CHILDREN'S NHS FOUNDATION TRUST	S	Gormley
		C	Kerrison
91	SHEFFIELD TEACHING HOSPITALS NHS FOUNDATION TRUST	Thushan	de Silva
		Simon	Tazzyman
92	SHERWOOD FOREST HOSPITALS NHS FOUNDATION TRUST	Lynne	Allsop
		Shrikant	Ambalkar
93	SHREWSBURY AND TELFORD HOSPITAL NHS TRUST	Mandy	Beekes
		Hannah	Gibson
94	SHROPSHIRE COMMUNITY HEALTH NHS TRUST	Johanne	Tomlinson
95	SOLENT NHS TRUST	Cathy	Price
		The Solent Research Team	
96	SOMERSET NHS FOUNDATION TRUST	Justin	Pepperell
		Kate	James
97	South Eastern Health & Social Care	Yuri	Protaschik
	South Eastern Health & Social Care	Tom	Trinick
98	SOUTHEND UNIVERSITY HOSPITAL NHS FOUNDATION TRUST	John	Day
		Swapna	Kunhunny
99	Southern Health & Social Care Trust	Angel	Boulos
		Alice	Neave
100	SOUTHERN HEALTH NHS FOUNDATION TRUST	Qi	Zheng

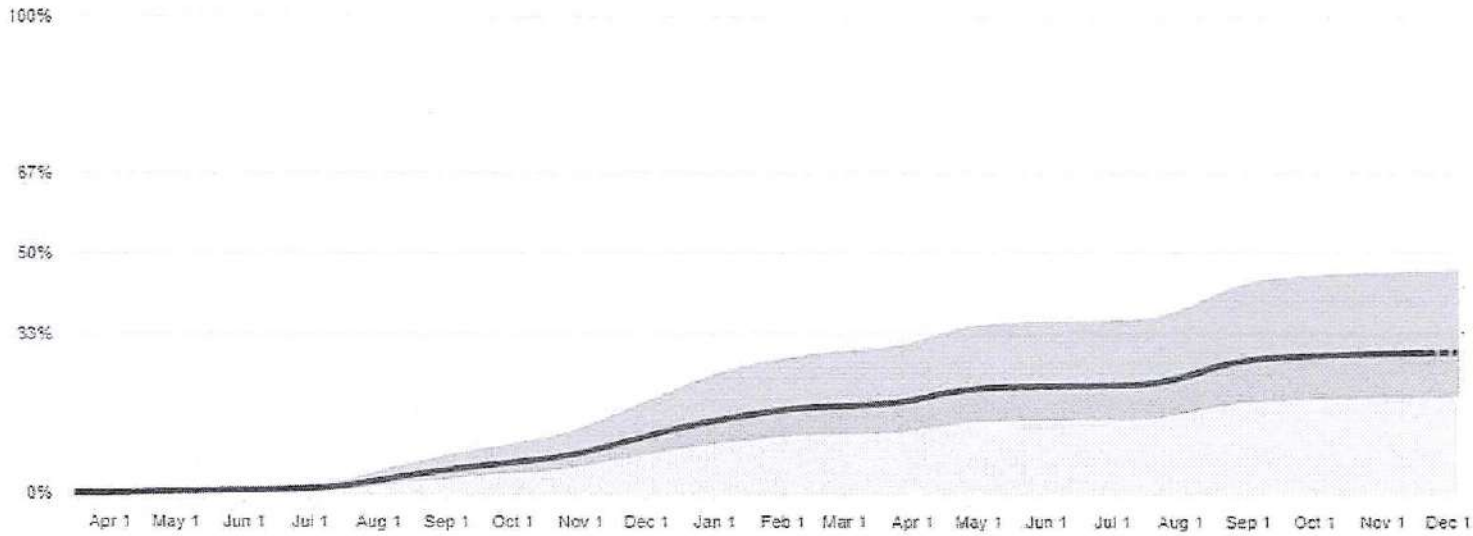
101	SOUTHPORT AND ORMSKIRK HOSPITAL NHS TRUST	Katherine	Gray
		Kerryanne	Brown
102	ST GEORGE'S UNIVERSITY HOSPITALS NHS FOUNDATION TRUST	Tim	Planche
		Angela	Houston
103	ST HELENS AND KNOWSLEY TEACHING HOSPITALS NHS TRUST	Rowan	Pritchard Jones
		Diane	Wycherley
104	STOCKPORT NHS FOUNDATION TRUST	Barzo	Faris
105	SURREY AND SUSSEX HEALTHCARE NHS TRUST	K	Nimako
		B	Stewart
106	Swansea Bay University LHB	Claire	Stafford
		Rebeccah	Thomas
107	THE CLATTERBRIDGE CANCER CENTRE NHS FOUNDATION TRUST	Sheena	Khanduri
		Nagesh	Kalakonda
108	THE DUDLEY GROUP NHS FOUNDATION TRUST	Helen	Ashby
109	THE HILLINGDON HOSPITALS NHS FOUNDATION TRUST	Natasha	Mahabir
110	THE NEWCASTLE UPON TYNE HOSPITALS NHS FOUNDATION TRUST	J	Harwood
		B	Payne
111	THE PRINCESS ALEXANDRA HOSPITAL NHS TRUST	Kathryn	Court
		Nikki	White
112	THE ROBERT JONES AND AGNES HUNT ORTHOPAEDIC HOSPITAL NHS FOUNDATION TRUST	Ruth	Longfellow
113	THE ROYAL BOURNEMOUTH AND CHRISTCHURCH HOSPITALS NHS FOUNDATION TRUST	Mihye	Lee
114	THE ROYAL WOLVERHAMPTON NHS TRUST	Marie	Green
		Lauren	Hughes
115	TORBAY AND SOUTH DEVON NHS FOUNDATION TRUST	Mathew	Halkes
		Pauline	Mercer
116	UNITED LINCOLNSHIRE HOSPITALS NHS TRUST	Alun	Roebuck
			ULHT Research Team
117	UNIVERSITY HOSPITAL SOUTHAMPTON NHS FOUNDATION TRUST	E	Wilson-Davies
118	UNIVERSITY HOSPITALS BRISTOL AND WESTON NHS FOUNDATION TRUST	Rajeka	Lazarus
		Aaran	Sinclair
119	UNIVERSITY HOSPITALS COVENTRY AND WARWICKSHIRE NHS TRUST	N	Aldridge
		L	Berry
120	UNIVERSITY HOSPITALS OF DERBY AND BURTON NHS FOUNDATION TRUST	F	Game
		T	Reynolds
121	UNIVERSITY HOSPITALS OF LEICESTER NHS TRUST	Christopher	Holmes
		Martin	Wiselka
122	UNIVERSITY HOSPITALS OF MORECAMBE BAY NHS FOUNDATION TRUST	Lynda	Fothergill
		Karen	Burns

123	UNIVERSITY HOSPITALS OF NORTH MIDLANDS NHS TRUST	Christopher	Duff
		Martin	Booth
124	UNIVERSITY HOSPITALS PLYMOUTH NHS TRUST	Hannah	Jory
		David	Hilton
125	Velindre NHS Trust	Charlotte	Young
		James	Powell
126	WALSALL HEALTHCARE NHS TRUST	Lisa	Richardson
		Aiden	Plant
127	WARRINGTON AND HALTON TEACHING HOSPITALS NHS FOUNDATION TRUST	Zaman	Qazzafi
		Lisa	Ditchfield
128	WEST SUFFOLK NHS FOUNDATION TRUST	A	Moody
		R	Tilley
129	Western Health & Social Care Trust	Tracy	Donaghy
		Maurice	O'Kane
130	WESTERN SUSSEX HOSPITALS NHS FOUNDATION TRUST	R	Sierra
		K	Shipman
131	WHITTINGTON HEALTH NHS TRUST	Philippa	Kemsley
132	WIRRAL UNIVERSITY TEACHING HOSPITAL NHS FOUNDATION TRUST	D	Harvey
		Y	Huang
133	WYE VALLEY NHS TRUST	L	Robinson
134	YEOVIL DISTRICT HOSPITAL NHS FOUNDATION TRUST	Sarah	Board
		Andrew	Broadley
135	YORK TEACHING HOSPITAL NHS FOUNDATION TRUST	Claire	Brookes
		Mags	Szewczyk
No.	**SIREN Associated Studies**	**First name**	**Surname**
1.	Protective Immunity from T cells to Covid-19 in Health workers (PITCH)	Susie	Dunachie
2.	Protective Immunity from T cells to Covid-19 in Health workers (PITCH)	Paul	Klenerman
3.	Protective Immunity from T cells to Covid-19 in Health workers (PITCH)	Chris	Duncan
4.	Protective Immunity from T cells to Covid-19 in Health workers (PITCH)	Lance	Turtle
5.	Protective Immunity from T cells to Covid-19 in Health workers (PITCH)	Alex	Richter
6.	Protective Immunity from T cells to Covid-19 in Health workers (PITCH)	Thushan	De Silva
7.	Protective Immunity from T cells to Covid-19 in Health workers (PITCH)	Eleanor	Barnes
8.	Protective Immunity from T cells to Covid-19 in Health workers (PITCH)	Daniel	Wootton
9.	The Humoral Immune Correlates for COVID-19 (HICC) consortium	Jonathan	Heeney
10.	The Humoral Immune Correlates for COVID-19 (HICC) consortium	Helen	Baxendale

11.	The Humoral Immune Correlates for COVID-19 (HICC) consortium	Javier	Castillo-Olivares
12.	The Francis Crick Institute	Rupert	Beale
13.	The Francis Crick Institute	Edward	Carr
14.	Genotype2Phenotype (G2P)	Wendy	Barclay
15.	Genotype2Phenotype (G2P)	Massimo	Palmarini
16.	GenOMICC	John Kenneth	Baillie

Percent Ever Infected

Percent ever infected is our estimate of the number of individuals in the county or state population who have been infected at least once with COVID-19.



Model input data

The case and death data used in the latest model run, and the 7-day moving average. Retrospective edits to this data are common to correct previous errors. These edits (and the errors that precede them) can influence our estimates a lot. You can use the dropdown to inspect archived model input data to see if this may be the case.

Data Table for Cumulative COVID-19 Nucleic Acid Amplification Tests (NAATs) Performed per 100k by State/Territory

CDC | Data as of: December 5, 2021 1:11 PM ET. Posted: December 5, 2021 2:23 PM ET

State	Cumulative Tests Performed per 100K
Rhode Island	513,570.864
Massachusetts	476,704.638
District of Columbia	466,790.588
Vermont	434,088.878
Alaska	413,735.882
New York*	340,029.124
Connecticut	335,200.981
Minnesota	314,647.426
Delaware	292,792.526
Maryland	264,330.538
North Dakota	262,591.45
Illinois	251,303.123
California	246,566.764
Maine	228,498.727
New Jersey	226,040.085
West Virginia	225,817.759
Wisconsin	223,111.482
New Hampshire	216,842.656
New Mexico	205,412.95
Colorado	205,111.731
Florida	202,506.53
Wyoming	199,401.368
Michigan	193,766.452
Louisiana	191,379.471
South Carolina	190,585.028
Hawaii	188,462.523
Utah	178,009.735
Indiana	170,103.656
North Carolina	164,724.56
Pennsylvania	163,088.123
Montana	158,377.978
Arizona	157,084.976
Kentucky	156,010.15
Iowa	151,316.57
Ohio	151,302.101
Nevada	150,461.992
Missouri	149,730.604
Kansas	149,473.935
Idaho	138,205.158
Oregon	136,172.285
Virginia	134,619.535
Tennessee	134,531.688
Texas	133,327.987
Alabama	128,021.925
Arkansas	127,749.774
Nebraska	127,062.035
Washington	125,432.983
Georgia	116,604.689
Guam	113,851.578
South Dakota	100,347.479
Virgin Islands	90,485.995
Oklahoma	81,461.738
Mississippi	68,553.714
Puerto Rico	58,488.838
American Samoa	N/A
Federated States of Micronesia	N/A
New York (Level of Community Transmission)*	N/A
New York City*	N/A
Northern Mariana Islands	N/A
Palau	N/A
Republic of Marshall Islands	N/A

Footnotes

CDC, United States COVID-19 Cases, Deaths, and Laboratory Testing (NAATs) by State, Territory, and Jurisdiction (View: Tests Performed, Time period: All Time, Metric: Rate per 100,000), *Data Table for Cumulative COVID-19 Nucleic Acid Amplification Tests (NAATs) Performed per 100k by State/Territory*, https://covid.cdc.gov/covid-data-tracker/#cases_testsper100k

Exh 28

PLAINTIFF'S EXHIBIT 30 21-1411(RAM) 12/7/2021

PENGAD 800-631-6989

HIGH PROFILE I Laboratory

Salus (Antigua Clinica Las Americas Guaynabo)
Tels. 787-720-0760
787-720-0767 / Fax 787-720-0763
Director: Lcda. Carmen Ripoll Lic. No. 1081 CLIA # 40D1027307

ANALYSIS REPORT

Patient Number	Birthdate	Sex	Page
355951	[redacted]	F	1

Physician Name
Dr. MORALES RUIZ, CARLOS

Request	Referent
OTERO SANTIAGO, OFELIA [redacted] TOA ALTA,PR 00953	

Specimen Obtained	Director
Nov/05/2020 10:58:41 am	CRM

Reported On	M.T.
Nov/05/2020 11:20:41 am	MRR/5964

Test	Units	Results Graphic	Normal Range

Molecular Test

SARS-CoV-2 RNA by ID NOW

SARS-CoV-2 RNA		POSITIVE	NEGATIVE

Notes

- METHOD: ID NOW COVID 19 is an automated assay that utilizes isothermal nucleic acid amplification technology for the qualitative detection of SARS-CoV-2 viral RNA.

Interpretación:

Positive: Positive results do not rule out bacterial infection or co-infection with other viruses.
Negative: Negative results should be treated as presumptive and, if inconsistent with clinical signs and symptoms or necessary for patient management should be tested with an alternative molecular assay. A negative result does not rule out co-infections with other pathogens.

This test has been authorized by FDA under an Emergency Use Authorization for use by authorized laboratories.

HIGH PROFILE | Laboratory

Salus (Antigua Clinica Las Americas Guaynabo)
Tels. 787-720-0760
787-720-0767 / Fax 787-720-0763
Director: Lcda. Carmen Ripoll Lic. No. 1081 CLIA # 40D1027307

ANALYSIS REPORT

Patient Number	Birt		
400742	[redacted]	F	1

Physician Name
Dr. PRIVADO, MEDICO

Request	Referent
OTERO SANTIAGO, OFELIA [redacted]	

Specimen Obtained	Director
Aug/13/2021 10:02:23 am	CRM

Reported On	M.T.
Aug/13/2021 11:12:04 am	NGL/7486

Test	Units	Results Graphic	Normal Range
Serology test			
by CareStart COVID-19 Antigen test			
COVID-19 Antigen		NEGATIVE	NEGATIVE

Notes

- This test has not been FDA cleared or approved.

  This test has been authorized by FDA under an EUA for use by authorized laboratories.

  This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or of COVID-19.

HIGH PROFILE I Laboratory
Salus (Antigua Clinica Las Americas Guaynabo)
Tels. 787-720-0760
787-720-0767 / Fax 787-720-0763
Director: Lcda. Carmen Ripoll Lic. No. 1081 CLIA # 40D1027307

ANALYSIS REPORT

Patient Number	Birthdate	Sex	Page
402272	[redacted]	F	1

Physician Name

Dr. MORALES RUIZ, CARLOS

Request	Referent
OTERO SANTIAGO, OFELIA [redacted] TOA ALTA,PR 00953	

Specimen Obtained	Director
Aug/20/2021 11:17:43 am	CRM

Reported On	M.T.
Aug/20/2021 12:13:53 pm	NGL/7486

Test	Units	Results Graphic	Normal Range
Molecular Test			
SARS-CoV-2 RNA by ID NOW			
SARS-CoV-2 RNA	NEGATIVE		NEGATIVE

Notes

METHOD: ID NOW COVID 19 is an automated assay that utilizes isothermal nucleic acid amplification technology for the qualitative detection of SARS-CoV-2 viral RNA.

Interpretación:

Positive: Positive results do not rule out bacterial infection or co-infection with other viruses.
Negative: Negative results should be treated as presumptive and, if inconsistent with clinical signs and symptoms or necessary for patient management should be tested with an alternative molecular assay. A negative result does not rule out co-infections with other pathogens.

This test has been authorized by FDA under an Emergency Use Authorization for use by authorized laboratories.

COVID-19 Vaccination Record Card

CDC

Please keep this record card, which includes medical information about the vaccines you have received.

Por favor, guarde esta tarjeta de registro, que incluye información médica sobre las vacunas que ha recibido.

Olan Santiago — Last Name

Ofelia — First Name

MI

4/24/[redacted] — Date of birth

Patient number *(medical record or IIS record number)*

Vaccine	Product Name/Manufacturer / Lot Number	Date	Healthcare Professional or Clinic Site
1st Dose COVID-19	Pfizer FA7485	8/21/21 mm dd yy	Farmacia Yerina 1
2nd Dose COVID-19		/ / mm dd yy	
Other		/ / mm dd yy	
Other		/ / mm dd yy	

Bayamon Health Center
Calle Manuel F Rossi Esq Isabel Segunda
Bayamon, PR 00960-2759
(787)995-1900

BAYAMÓN HEALTH CENTER

Cap -
Joint Commission -
Health Lic - 1261
Clia 88 - 40D2038083

Nombre: OTERO SANTIAGO, OFELIA
Edad: 48 Años Género: Femenino
Dirección: [redacted]
TOA ALTA, PR 00953

Número de Cuenta: 0001385124 Récord: 0000423605
Fecha de orden: 08/27/2021
Médico: DR AMILCAR LUGO RIVERA
Procedimiento: ANTIGEN COVID19 TEST

PATIENT: OTERO SANTIAGO, OFELIA Record# 0000423605
DOB: [redacted] SEX: F Ordered by: LUGO RIVERA, AMILCAR E
Lab. Number: 349967 Date: 08/27/2021
Order Date: 08/27/2021 Order Time: 12:31:01
Collected Date: 08/27/2021 Collected Time: 12:31:00
Received Date: 08/27/2021 Received Time: 13:05:00
Processed Date: 08/27/2021 Processed Time: 13:27:00
Reported Date: Reported Time: : :
Processed by: LCDA. ELBA COSME ORTEGA
License: 1261 Clia: 40D2038083
Director: LCDA. NANCY JIMENEZ RODRIGUEZ License:LIC: 3

Test	Result	HL	R	Ref Range	Units
SARS-COV-2 ANTIGEN T					
COVID-19 ANTIGEN	NEGATIVE			NEGATIVE	

COMMENT
Method: Lateral Flow Immunoassay (BinaxNow COVID-19 Ag Card EUA)

A positive test result for COVID-19 indicates that antigens from SARS-CoV-2 were detected, and the patient is infected with the virus and presumed to

A negative test result means that antigens from SARS-CoV-2 were not present in the specimen above the limit of detection. However, a negative result does not rule out infection. If COVID-19 is still suspected, based on exposure history and clinical findings, testing with molecular methods should b

Antigen tests are known to be less sensitive than molecular tests. The amount of antigen in a sample may decrease as the duration of illness increases. Specimens collected after day 7 of illness may be more likely to be negative compared to a PCR assay. If COVID-19 is still suspected testing with mo

A negative antigen test should not be the sole basis used to determine if a

Bayamon Health Center
Calle Manuel F Rossi Esq Isabel Segunda
Bayamon, PR 00960-2759
(787)995-1900

Cap -
Joint Commission -
Health Lic - 1261
Clia 88 - 40D2038083

Nombre: OTERO SANTIAGO, OFELIA
Edad: 48 Años Género: Femenino
Dirección: [redacted]
TOA ALTA, PR 00953

Número de Cuenta: 0001385124 Récord: 0000423605
Fecha de orden: 08/27/2021
Médico: DR AMILCAR LUGO RIVERA
Procedimiento: COV-2 2019

PATIENT: OTERO SANTIAGO, OFELIA Record# 0000423605
DOB: [redacted] SEX: F Ordered by: LUGO RIVERA, AMILCAR E
Lab. Number: 349967 Date: 08/27/2021
Order Date: 08/27/2021 Order Time: 12:31:01
Collected Date: 08/27/2021 Collected Time: 12:31:00
Received Date: 08/27/2021 Received Time: 13:05:00
Processed Date: 08/27/2021 Processed Time: 13:28:00
Reported Date: Reported Time: : :
Processed by: LCDA. ELBA COSME ORTEGA
License: 1261 Clia: 40D2038083
Director: LCDA. NANCY JIMENEZ RODRIGUEZ License:LIC: 3

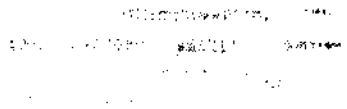
Test	Result	HL	R	Ref Range	Units
COVID19 IGG	POSITIVE			NEGATIVE	
COVID19 IGM	NEGATIVE			NEGATIVE	

COMMENT
Result by HEALGEN COVID-19 IgG/IgM Rapid Test:

- This test has not been reviewed by FDA.

- Negative results do not rule out SARS-CoV-2 infection, particularly in th
ose who have been in contact with the virus. Follow-up testing with a molec
ular diagnostic should be considered to rule out infection in these individ

- Results from antibody testing should not be used as the sole basis to dia



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Bayamon, PR 00960-2759
(787)995-1900

Cap -
Joint Commission -
Health Lic - 1261
Clia 88 - 40D2038083

Nombre: OTERO SANTIAGO, OFELIA
Edad: 48 Años Género: Femenino
Dirección: [redacted]
TOA ALTA, PR 00953

Número de Cuenta: 0001385124 Récord: 0000423605
Fecha de orden: 08/27/2021
Médico: DR AMILCAR LUGO RIVERA
Procedimiento: CK OR CPK MB TOTAL

PATIENT: OTERO SANTIAGO, OFELIA Record# 0000423605
DOB: [redacted] SEX: F Ordered by: LUGO RIVERA, AMILCAR E
Lab. Number: 349972 Date: 08/27/2021
Order Date: 08/27/2021 Order Time: 14:41:00
Collected Date: 08/27/2021 Collected Time: 14:41:00
Received Date: 08/27/2021 Received Time: 15:17:00
Processed Date: 08/27/2021 Processed Time: 15:18:00
Reported Date: Reported Time: : :
Processed by: LCDA. ELBA COSME ORTEGA
License: 1261 Clia: 40D2038083
Director: LCDA. NANCY JIMENEZ RODRIGUEZ License:LIC: 3

Test	Result	HL	R	Ref Range	Units
CPK- TOTAL	76			26 - 192	U/L
CK-MB	18			0 - 25	U/L
MB/CK RATIO	23				

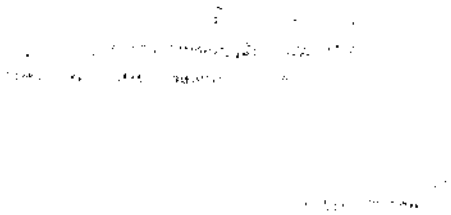
COMMENT
NEW CKMB INTERPRETATION:

THERE IS HIGH PROBABILITY OF MYOCARDIAL DAMAGE WHEN THE FOLLOWING THREE CON

1. CK(MEN) >195 U/L OR CK(WOMEN >170 U/L

2. CKMB >25 U/L

3. THE MB/CK RATIO ACTIVITY ACCOUNTS FOR 6-25% OF THE TOTAL CK ACTIVITY.



Bayamon Health Center
Calle Manuel F Rossi Esq Isabel Segunda
Bayamon, PR 00960-2759
(787)995-1900

Cap -
Joint Commission -
Health Lic - 1261
Clia 88 - 40D2038083

Nombre: OTERO SANTIAGO, OFELIA
Edad: 48 Años Género: Femenino
Dirección: [redacted]
TOA ALTA, PR 00953

Número de Cuenta: 0001385124 Récord: 0000423605
Fecha de orden: 08/27/2021
Médico: DR AMILCAR LUGO RIVERA
Procedimiento: BASIC METABOLIC PANEL

PATIENT: OTERO SANTIAGO, OFELIA Record# 0000423605
DOB: [redacted] SEX: F Ordered by: LUGO RIVERA, AMILCAR E
Lab. Number: 349967 Date: 08/27/2021
Order Date: 08/27/2021 Order Time: 12:31:01
Collected Date: 08/27/2021 Collected Time: 12:31:00
Received Date: 08/27/2021 Received Time: 13:05:00
Processed Date: 08/27/2021 Processed Time: 13:15:00
Reported Date: Reported Time: : :
Processed by: LCDA. ELBA COSME ORTEGA
License: 1261 Clia: 40D2038083
Director: LCDA. NANCY JIMENEZ RODRIGUEZ License:LIC: 3

Test	Result	HL	R	Ref Range	Units
BASIC METABOLIC PANE					
GLUCOSE	91			70 - 99	mg/dL
BUN	14			6 - 20.0	mg/dL
CREATININE	0.72			0.50 - 1.20	mg/dL
BUN/CREAT	19.4			10.0 - 20.00	
SODIUM (NA)	140			136 - 146	mmol/L
POTASSIUM (K)	4.46			3.5 - 5.1	mmol/L
CHLORIDE (CL)	102.3			98 - 108	mmol/L
CO2	25.0			22.0 - 29.0	mmol/L
ANION GAP	17.2			11 - 25.0	
CALCIUM	9.5			8.5 - 10.5	mg/dL
eGFR Non African	92			>60	
eGFR African America	111			>60	

COMMENT
The eGFR test is used to screen for detect early kidney damage and to monit
or kidney status. The estimated GFR (eGFR) is reported in two categories:
eGFR non-African American and eGFR African American. The MDRD equation has
not been validated in children <18 years of age, pregnant women, patients
older than 70 years, or racial or ethnic subgroups other than Caucasians

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Nombre: OTERO SANTIAGO, OFELIA
Edad: 48 Años Género: Femenino
Dirección: [redacted]
TOA ALTA, PR 00953

Número de Cuenta: 0001385124 Récord: 0000423605
Fecha de orden: 08/27/2021
Médico: DR AMILCAR LUGO RIVERA
Procedimiento: CBC W DIFF

%

INMATURE
NRBC
PLTS
RBC MORPHOLOGY

COMMENT

Bayamon Health Center
Calle Manuel F Rossi Esq Isabel Segunda
Bayamon, PR 00960-2759
(787)995-1900

Cap -
Joint Commission -
Health Lic - 1261
Clia ## - 40D2038083

Nombre: OTERO SANTIAGO, OFELIA
Edad: 48 Años Género: Femenino
Dirección: [redacted]
TOA ALTA, PR 00953

Número de Cuenta: 0001385124 Récord: 0000423605
Fecha de orden: 08/27/2021
Médico: DR AMILCAR LUGO RIVERA
Procedimiento: CBC W DIFF

PATIENT: OTERO SANTIAGO, OFELIA Record# 0000423605
DOB: [redacted] SEX: F Ordered by: LUGO RIVERA, AMILCAR E
Lab. Number: 349967 Date: 08/27/2021
Order Date: 08/27/2021 Order Time: 12:31:01
Collected Date: 08/27/2021 Collected Time: 12:31:00
Received Date: 08/27/2021 Received Time: 13:05:00
Processed Date: 08/27/2021 Processed Time: 13:17:00
Reported Date: Reported Time: : :
Processed by: LCDA. ELBA COSME ORTEGA
License: 1261 Clia: 40D2038083
Director: LCDA. NANCY JIMENEZ RODRIGUEZ License:LIC: 3

Test	Result	HL	R	Ref Range	Units
COMPLETE BLOOD COUNT					
WBC	4.75		L	4.8	10.8	10^3/uL
RBC	4.44		L	4.70	6.10	10^6/uL
HEMOGLOBIN	13.5			12.0	16.0	g/dL
HEMATOCRIT	39.9			37.0	47.0	%
MCV	90.0			81	99	fL
MCH	30.4			27.0	31.0	pg
MCHC	33.8			33.0	37.0	g/dL
RDW	10.9		L	11.5	14.5	%
PLATELETS	226			130	400	10^3/uL
MPV	10.9		H	7.4	10.4	fL
DIFFERENTIAL						
NEUT%	53.4			42.2	75.2	%
LYM% (LYMPHS)	37.4			20.5	51.1	%
MONO%	4.9			1.7	9.3	%
EOS%	4.0		H	0.0	3.0	%
BASO%	0.3			0.0	3.0	%
NEUT #	2.54			1.4	6.5	10^3/uL
LYM #	1.78			1.2	3.4	10^3/uL
MONO #	0.23			0.1	0.6	10^3/uL
EOS #	0.19			0.0	0.7	10^3/uL
BASO #	0.01			0.0	0.1	10^3/uL
MANUAL DIFFERENTIAL						
SEG						%
LYMPHS						%
MONO						%
EOS						%
BASOS						%
ATYPICAL LYMPHOCYTES						%
BANDS						%
METAMYELOCYTE						%
MYELOCYTE						%
PROMYELOCYTE						%
BLAST						%

Universidad de Puerto Rico, University of Puerto Rico
Recinto de Ciencias Médicas, Medical Sciences Campus
Unidad de Medicina Comparada, Unit of Comparative Medicine

Carlos A. Sariol, MD, MS.
Director and Associate Professor

Virology and Microbiology Laboratory
University of Puerto Rico Medical Science Campus
Main Building Third Floor Room B329
San Juan, PR 00935
Lic. 1396

CLIA Waiver ID Number 40D2033432

Septiembre 29th, 2021

Name: Ofelia Otero Santiago
Sample ID: O-O 09/29/2021
Sample: 09-13-2021
Tested: 09-22-2021
Reported: 09-29-2021

COVID-19 IgG ELISA Test by CovIgG-Assay

COVID-19 IgG	**POSITIVE**	**Reference Value:** Negative
COVID-19 Antibody Titer	**8514**	**Reference Value:** 100 - 12800

Method: Quantitative Enzyme-linked Immunoassay (ELISA) for detection of SARS-CoV-2 infection.

INTERPRETATION OF RESULTS:
A positive IgG test result with the CovIgG-Assay indicates that antibodies to SARS-CoV-2 were detected, and the patient has potentially been exposed to COVID-19 or has received a COVID-19 vaccine.
A negative test result means that the antibodies to the virus that causes COVID-19 was not found in your sample.

All results must be interpreted together with other clinical information available to the physician.
If the test result is positive you may need isolation to avoid spreading the virus to others, or you may have been previously infected. Your healthcare provider will work with you to determine the best care for you based on the test results along with other factors of your medical history, symptoms, possible exposures, and geographic location of places you have recently traveled. There is also the small chance that this test can give a false positive result.

If test results are negative it is possible for this test to give a false negative result in some people with COVID-19. A negative result may occur if you are tested early in your illness and your body hasn't had time to produce antibodies to infection. This means that you could possibly still have COVID-19 even though the test is negative. If this is the case, your healthcare provider will consider the test result together with all other aspects of your medical history (such as symptoms, possible exposures, and geographical location of places you have recently traveled) in order to decide how to interpret these results.

This assay is intended for RESEARCH ONLY and NOT for the diagnosis, prevention, or treatment of any disease or impairment of, or the assessment of the health of, human beings.

CovIgG-Assay is not cleared; CLIA waived, approved, or subject to an approved investigational device exemption. This assay was submitted to FDA for Emergency Use Authorization (Submission Number EUA201115).
https://prsciencetrust.org/the-coviqm-assay-kit/

About this space

Hillside Cabin, is a modern tropical and safe container (wagon) made as a mini h
isolated, it has 1 and a half ropes of land, above one of many of the highest peaks of the mountainous city of Mayaguez, PR. It offers a spectacular panoramic view, scenic landscapes of mountains and sea. It is located several minutes from the village of Mayagüez, Cape Red, Anasco and Rincon. Close to beaches and rivers.

The space

It is a rope and a half of private land, when entering through the gate of the room we have neighbors, and main road, when going down we have 220 feet from the entrance to the room, when you arrive at Hillside Cabin, as such we have no neighbors around it, only nature and at the end of the land there is the fire area and the view to the mountains and the sea.
The room has a fourth queen bed with its linens, air conditioning.
Living room with furniture that becomes a bed, (full) and air conditioning.
Bathroom with soap, shampoo, conditioner, towels, toilet paper, solar heater.
Kitchen with your utensils, Nespresso coffee maker, Greek for coffee, kettle, microwave, gas stove, refrigerator, toaster, we leave you, sugar, tea, cremora, coffee, 4 bottles of water.

The patio area we have Bbq (gas), patio dining table, rocking bed, hot jacuzzi and on the terrace have pool.
Controlled access, security cameras, modern and tropical decor, smart TV we have wifi and Netflix.
Designed for guests to have a spiritual retreat, meditation, relaxation, in the countryside only with the sky of the day and the stars in the night and coqui company.

And the best part of all the stay is completely private just for you!!!

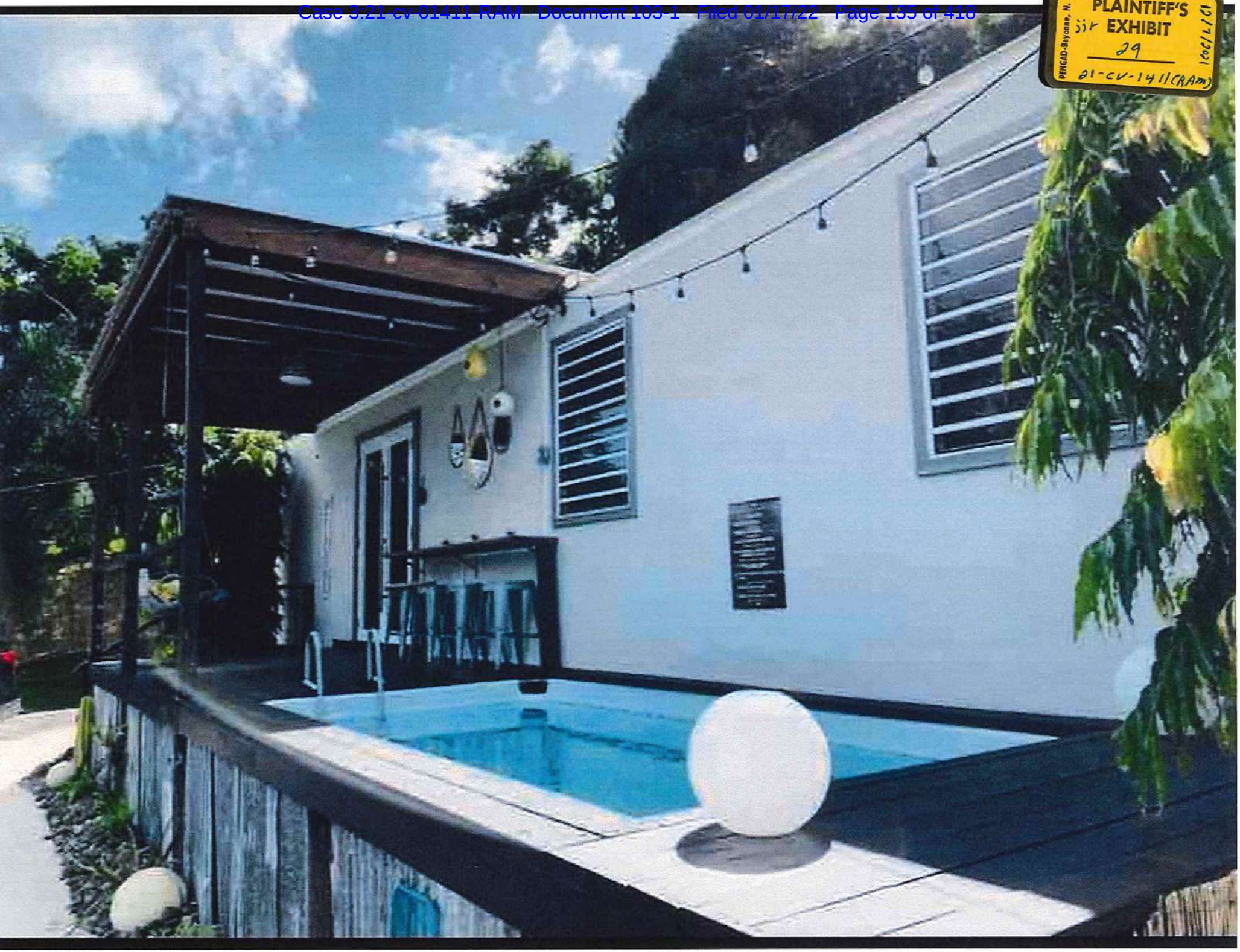
Guest access

It has a hammock, hanging bed, jacuzzi and pool and a bbq area and relaxation by the greenery of ground rope. More view of the mountains and the sea.

Other things to note

At Hillside Cabin we do not accept activities, nor groups of people who are not registered in the stay, with tamos with security cameras at the entrance and in the parking areas of the stay and in our warehouse, the cameras never evade your privacy.
We have our warehouse closed is for the use of owners and employees only, it is forbidden to enter that area, which has an alarm and security cameras. If you enter the restricted area you will have a charge of $200.00 for entering the warehouse.
Pets are prohibited.

*If they don't follow the rules they have to leave the stay without a refund.



3960 RENE L. MATOS RUIZ

	Hours	Rate	Total		This Check	Year to Date
Regular	40.00	8.00	320.00	Gross	320.00	11,538.15
TimeandHalf		12.00		Soc_Sec	-19.84	-715.34
Vacation		8.00		Medicare	-4.64	-167.34
Sick		8.00		SDI		-27.00
EFMLEA		8.00		ChildSupor	-104.40	-4,339.50

40.00 320.00 $191.12 103056

Check Number: 103056 Oct 26, 2021

SUPERMERCADO ECONO
AGUEYBANA PO BOX 3009
YAUCO, PR 00698

191.12

3960 RENE L. MATOS RUIZ xxx-xx-[redacted] 10/25/21 10/31/21

	Hours	Rate	Total		This Check	Year to Date
Regular	36.00	8.00	288.00	Gross	288.00	11,826.15
TimeandHalf		12.00		Soc_Sec	-17.86	-733.20
Vacation		8.00		Medicare	-4.18	-171.52
Sick		8.00		SDI		-27.00
EFMLEA		8.00		ChildSupor	-104.40	-4,443.90

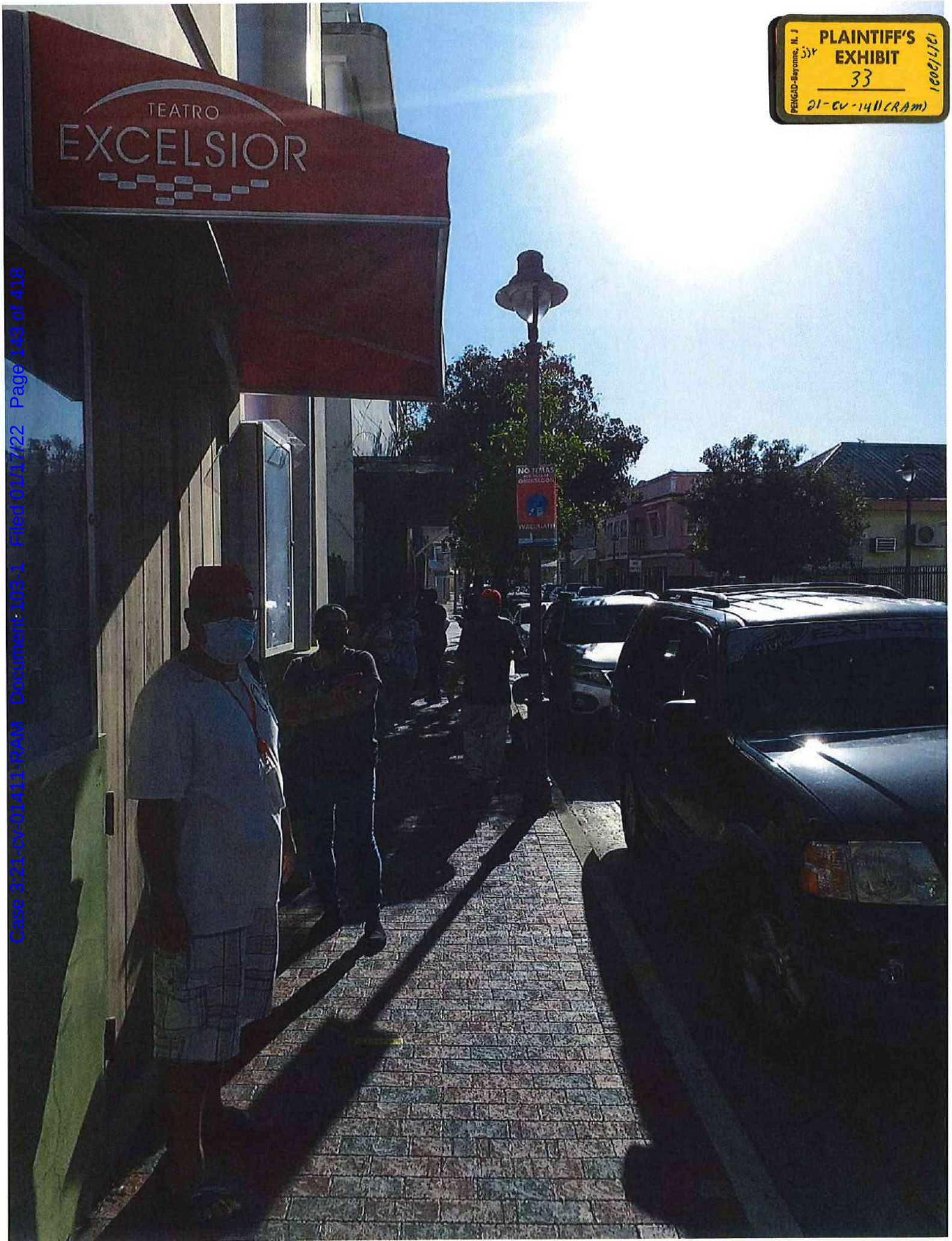
36.00 288.00 $161.56 103153

Check Number: 103153 Oct 31, 2021

SUPERMERCADO ECONO
AGUEYBANA PO BOX 3009
YAUCO, PR 00698

3960 RENE L. MATOS RUIZ xxx-xx-[redacted] 11/1/21 11/7/21

	Hours	Rate	Total		This Check	Year to Date
Regular	32.00	8.00	256.00	Gross	256.00	12,082.15
TimeandHalf		12.00		Soc_Sec	-15.87	-749.07
Vacation		8.00		Medicare	-3.71	-175.23
Sick		8.00		SDI		-27.00
EFMLEA		8.00		ChildSupor	-104.40	-4,548.30



EXCELSIOR
PENGAD-Bayonne, N. J.
PLAINTIFF'S EXHIBIT
30
21-CV-1411(RAM)
1/6/2022

NDPTC
NATIONAL DISASTER PREPAREDNESS TRAINING CENTER
UNIVERSITY of HAWAI'I

FEMA

National Disaster Preparedness Training Center

In collaboration with
Department of Homeland Security
Federal Emergency Management Agency

Certificate of Completion Presented To

Eliza Llenza

for successfully completing

Planning for Disaster Debris Management

MGT-460

AUTHORIZED IACET PROVIDER

(8 hours, 0.6 CEU)

on this day
November 13, 2019

Karl Kim, Executive Director
National Disaster Preparedness Training Center

Atlantic OSHA Training Center
An Authorized OSHA Training Institute Education Center®

UNIVERSIDAD ANA G. MÉNDEZ
ESCUELA DE CIENCIAS, TECNOLOGÍA Y AMBIENTE
INSTITUTO DE EDUCACIÓN AMBIENTAL

This is to certify that

Eliza Llenza

Has successfully completed the course

Mold Clean-Up and Safety after Disasters

March 13, 2019

María C. Ortiz
Associate Dean
School of Science, Technology & Environment

INEDA/AOTC, PR Región II
Bayamón, Puerto Rico

Rafael A. Caballero Torres
Director of INEDA
School of Science, Technology & Environment

CERTIFIED PROFESSIONAL FOOD MANAGER

Designation Has Been Conferred Upon

ELIZA LLENZA

PROMETRIC

Who has met all the professional requirements for certification in food service safety and sanitation.

ANSI ACCREDITED PROGRAM American National Standards Institute and the Conference for Food Protection #0659

Exam 4811 Recognized By Conference For Food Protection

Certificate No: 2080839
Exam Date: 12/07/19

Test Code: 6203064811
Expires on: 12/07/24

Ryan M. McMillion

Ryan McMillion, Client Services Manager

Prometric | 7941 Corporate Drive, Nottingham, MD 21236 | 800.624.2736

Cut Here

Prometric Score Report

Congratulations! You passed the Certified Professional Food Manager examination.

Your Score is as follows:

Score	Status	Exam Date
92	PASS	12/07/2019

PROMETRIC This is to certify that

ANSI #0659

ELIZA LLENZA

Has met the necessary requirements for

FOOD MANAGER CERTIFICATION

Exam 4811 Recognized By Conference For Food Protection

ID # 009371974 Exam Date 12/07/19

Cert # 2080839 Expires On 12/07/24

Cut Here

CMTAS

ELIZA LLENZA
1713 CALIFORNIA ST
SAN JUAN, PR 00926

Neha - Bosque

LABORATORIO CLINICO COSTA CARIBE

Laboratorio Clinico Costa Caribe - Cupey
Carr. 844 Numero 1749 Urb. Purple Tree
Cupey, PR 00926
Phone: (787) 748-4848 Fax: (787) 748-4008
CLIA: 40D0667626 LICENSE NO.: 661

Patient ID: 661/ 14204
Patient Address: Elisa Llenza Zuecca
[redacted]
Visit Date: 27-Dec-20
Ordered By: LOPEZ NIEVES ZOILO M.D.
Record Number: 3732-661
Stamp (CTMPR): No Digital Stamp
Cost per stamp: No Digital Stamp
Administrative

5¢ A3570808 5¢

SP Number: 12 - 75647
Customer Sex: Female
Home Telephone: [redacted]
Work Telephone:
Fax Number:
Birthday: [redacted]
Age: 63 - 08

TEST DESCRIPTION	RESULT	NORMAL VALUES	MT
SARS-CoV-2 Antigen	POSITIVE	NEGATIVE	SAR

This test has not been FDA cleared or approved;this test has been authorized by FDA under an EUA for use by authorized laboratories;certified under the CLIA.Results should be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient. False negative results may occur in patients who have indicated taking high doses of Biotin (>10 mg per day)

R = Rechecked D = Rechecked by Dilution
Comments :

Visit Date / Time / By :	Sample Taken Date / Time :	Reported Date / Time / By :	Printed Date / Time / By :	MT
12/27/2020 8:51:29 AM SECRETARIA ENTRENAMIENT	12/27/2020 8:51:29 AM	12/27/2020 11:21:45 AM SAR	12/27/2020 11:21:48 AM LCDA. SELINES ALVARADO R	SAR

Laboratory Report

Immuno Reference Lab
AVE. MUÑOZ RIVERA #562 SAN JUAN, PUERTO RICO 00919
TEL. (787) 999-2990 / FAX: (787) 764-8809
CLIA 40D0658269 / LIC 268 - CLIA 40D0658265 / LIC 338
www.immunopr.com

IMRL CLINICAL LAB HATO REY
LAB-00192
Phone : (787)296-9997
Fax : (787)296-9998

PATIENT NAME		ACCESSION NO.	AGE	SEX	NATIONAL LAB ID
LLENZA ZUECCA, ELISA		043GZHBZ	64Y	F	96441
DR. MARTINEZ RIVERA LUIS	ROOM	[redacted]	Jun/26/2021		Jun/28/20 DATE

TEST	RESULT		NORMAL VALUE
SARS-CoV-2 IgM Qly & IgG Qty			
SARS-CoV-2 IgM assay	NOT-DETECTED		NOT DETECTE
SARS-CoV-2 IgG II, Semi-Quantitative	83.85	AU/mL	

Notes

Method: Chemiluminescent Immunoassay since March 8, 2021.

This test has not been reviewed by the FDA.

LESS THAN 10 AU/mL SARS-CoV-2 IgG II = NOT DETECTED
GREATER OR EQUAL 10 AU/mL SARS-CoV-2 IgG II = DETECTED

The Access SARS-CoV-2 IgM, IgG S Spike assay is intended for use as an aid in identifying patients with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The Access SARS-CoV-2 IgM assay should not be used to diagnose or exclude acute SARS-CoV-2 infection.

Results are for the detection of SARS-CoV-2 antibodies. IgM antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Patients may have detectable virus present for several weeks following seroconversion.

IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Patients may have detectable virus present for several weeks following seroconversion.

Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

Results from antibody testing should not be used to diagnose or exclude acute SARS-CoV-2 infection.

Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.

Colegio de Tecnologos Medicos de PR - Patente 0000348
Paid 5 Cents - Expiration Date:February/28/2022 - Num 2021-015605700

Originally Reported Jun/26/2021 12:03:17 am
Tests Performed by SA2 Lic# 7403
Medical Technologist

IMRL
Immuno Reference Lab
AVE. MUÑOZ RIVERA #562 SAN JUAN, PUERTO RICO 00919
TEL. (787) 999-2990 / FAX: (787) 764-8809
CLIA 40D0658269 / LIC 268 - CLIA 40D0658265 / LIC 338
www.immunopr.com

21 IMRL Immuno Reference Lab ANNIVERSARY

Laboratory Report

IMRL CLINICAL LAB HATO REY
LAB-00192
Phone : (787)296-9997
Fax : (787)296-9998

PATIENT NAME		MEDICAL RECORD NO.	AGE	SEX	NATIONAL LAB ID
LLERENZA ZUECCA, ELISA		0491HAMT	64Y	F	98344
PHYSICIAN	ROOM	BIRTHDATE	RECEIVED DATE		PRINTED DATE
DR. MARTINEZ RIVERA LUIS	/	May/13/1957	Sep/16/2021		Sep/17/2021

TEST	RESULT	NORMAL VALUE
SARS-CoV 2 IgG S-SPIKE Semi-QTY		
SARS-CoV-2 IgG II, Semi-Quantitative	46.59 AU/mL	

Notes

Method: Chemiluminescent Immunoassay since March 8, 2021.

This test has not been reviewed by the FDA.

LESS THAN 10 AU/mL SARS-CoV-2 IgG II = NOT DETECTED
GREATER OR EQUAL 10 AU/mL SARS-CoV-2 IgG II = DETECTED

The Access SARS-CoV-2 IgM, IgG S Spike assay is intended for use as an aid in identifying patients with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The Access SARS-CoV-2 IgM assay should not be used to diagnose or exclude acute SARS-CoV-2 infection.

The results of this semi-quantitative test should not be interpreted as an indication or degree of immunity or protection from reinfection.

Results are for the detection of SARS-CoV-2 antibodies. IgM antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Patients may have detectable virus present for several weeks following seroconversion.

IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Patients may have detectable virus present for several weeks following seroconversion.

Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.

Maria E. Carrascal Muñoz

Shell Castle #22• Humacao, P.R., Palmas INN Way, Suite 130, PMB 322
Phone: (787) 644 - 7816 • Fax: (787) 656 - 8706 • E-Mail: dreamwrks@gmail.com

Education

University of Puerto Rico (UPR), Río Piedras, BS Biology Magna Cum Laude (1982 -1986).

Temple University, Intensive English Language Course (1985).

San Cristopher Hospital, Temple Universiy Philadelphia, Pediatric Nephrology Internship (1989).

M.D. Universidad Central del Caribe UCCEM School of Medicine, HURRA, Bayamón, Puerto Rico (1990).

Graduated Specialty in Pediatrics (1993) from: U.M.D.N.J. Rutgers University, NJ Children Hospital of New Jersey, Trauma Center Level I.

Graduated Subspecialty in Pediatric Infectious Diseases, Allergy & Immunology (1995) from: U.M.D.N.J. Rutgers University N.J. National Institutes of Health Research Center, National HIV Resource Center, N.J.

Experience

. CDT Suzana Centeno, Vieques 2017 – currently.

. Antibiotic Stewardship Medical Expert (Adult and Pediatric population), Menonita, Caguas, 2017- currently

- Attending Physician of U.P.R. Recinto de Río Piedras, Dr. López Nussa, HMSJ
  - Ciencias Médicas, Medical School and Pediatric Residency Program since 1996 – 2021.
- Pediatric Infectious Diseases Consultant of:
  - San Francisco Metro Pavía, Health System, currently.
- Dr. Lopez Nussa, HMSJ
* Pediatric Residency Program
* HIMA San Pablo, Caguas (2004- 2018)
* Pediatrics Continuity Care Clinic for Dr. Lopez Nussa Pediatric Residency Program (2000 – 2004).

- President of Infection Control Committee Hospital San Francisco Metro Pavía (2011-2014).
- President of Infectious Control Committee, HMSJ (1998-2005).
- Humanitarian Missions A.M.A.R. (Alianza de Médicos al Rescate) since 1998 – currently.
- Medico – Legal Expert SIMED (since 1996 – currently)
- Pediatric Functional Medicine Practice.
- Nutrition and Agroecological approach to health practice.
- Medical Students, College Mentorship.

Publications/Presentations

- Education articles in in Health magazine.
- Conferenciante y catedrática de Sociedad de Infectólogos de Puerto Rico, Academia de Pediatria de P.R, AMPRE, AMPRO, Sociedad de Médicos tratantes de HIV, entre otros.
- Author of the DVD: Como Ayude a mi hijo con Déficit de Atención.
- Incidence of Tuberculosis in the Pediatric Population (2006)
- Correlation of Neurodevelopmental Delays and Dysbiosis in HIV Exposed pediatric population. (2017).

Research Experience

- Pediatric Subspecialty Clinic HIV exposed and HIV infected population treatment and follow up care (2011-2017).
- Integrative Center for Special Pediatric Population
- Multicenter Study Use of Linezolid in Pediatrics (1999)

Teaching Experience

- Attending Physician of U.P.R. Recinto de Río Piedras, Dr López Nussa, HMSJ
  - ➢ Ciencias Médicas, Medical School and Pediatric
  - ➢ Residency Program since 1996 - 2021.
- Pediatric Infectious Diseases Consultant of San Francisco Metro Pavía, Health System, Dr. Lopez Nussa, HMSJ, Hospital Menonita, Caguas.
- Pediatric Residency Program, HIMA, Caguas
- Professor of clinical Homeopathy C.E. D. H. (Since 2004- 2013).

* Pediatrics Continuity Clinics of HMSJ (2000-2004).

* Pediatric care at CDT Susana Centeno, Vieques 2017 – currently.

- Mentorship Program for medical students (currently).
- Pediatrics and Family Medical Integrative and Functional Medicine Practice.

Professional Development

- Doctors Choice Award Pediatric Infectious Diseases, Allergy Immunology and Functional Medicine, Buena Vida 2014 and subsequent years.

Affiliations/Memberships

- American Academy of Anti – Aging Medicine A4M.
- American Academy of Pediatrics Chapter of Puerto Rico.
- Infectious Diseases Society of Puerto Rico.
- Co Founder of Centro de Referncia para Autismo y Neurodesarrollo. (C.R.A.N.)
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Title page

Comparing SARS-CoV-2 natural immunity to vaccine-induced immunity: reinfections versus breakthrough infections

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Abstract

Background:

Reports of waning vaccine-induced immunity against COVID-19 have begun to surface. With that, the comparable long-term protection conferred by previous infection with SARS-CoV-2 remains unclear.

Methods:

We conducted a retrospective observational study comparing three groups: (1)SARS-CoV-2-naïve individuals who received a two-dose regimen of the BioNTech/Pfizer mRNA BNT162b2 vaccine, (2)previously infected individuals who have not been vaccinated, and (3)previously infected *and* single dose vaccinated individuals. Three multivariate logistic regression models were applied. In all models we evaluated four outcomes: SARS-CoV-2 infection, symptomatic disease, COVID-19-related hospitalization and death. The follow-up period of June 1 to August 14, 2021, when the Delta variant was dominant in Israel.

Results:

SARS-CoV-2-naïve vaccinees had a 13.06-fold (95% CI, 8.08 to 21.11) increased risk for breakthrough infection with the Delta variant compared to those previously infected, when the first event (infection or vaccination) occurred during January and February of 2021. The increased risk was significant ($P<0.001$) for symptomatic disease as well. When allowing the infection to occur at any time before vaccination (from March 2020 to February 2021), evidence of waning natural immunity was demonstrated, though SARS-CoV-2 naïve vaccinees had a 5.96-fold (95% CI, 4.85 to

7.33) increased risk for breakthrough infection and a 7.13-fold (95% CI, 5.51 to 9.21) increased risk for symptomatic disease. SARS-CoV-2-naïve vaccinees were also at a greater risk for COVID-19-related-hospitalizations compared to those that were previously infected.

Conclusions:

This study demonstrated that natural immunity confers longer lasting and stronger protection against infection, symptomatic disease and hospitalization caused by the Delta variant of SARS-CoV-2, compared to the BNT162b2 two-dose vaccine-induced immunity. Individuals who were both previously infected with SARS-CoV-2 and given a single dose of the vaccine gained additional protection against the Delta variant.

Introduction

The heavy toll that SARS-CoV-2 infection has been taking on global health and healthcare resources has created an urgent need to estimate which part of the population is protected against COVID-19 at a given time in order to set healthcare policies such as lockdowns and to assess the possibility of herd immunity.

To date, there is still no evidence-based, long-term correlate of protection[1]. This lack of correlate of protection has led to different approaches in terms of vaccine resource allocation, namely the need for vaccine administration in recovered patients, the need for booster shots in previously vaccinated individuals or the need to vaccinate low-risk populations, potentially previously exposed.

The short-term effectiveness of a two-dose regimen of the BioNTech/Pfizer BNT162b2 mRNA COVID-19 vaccine was demonstrated in clinical trials[2] and in observational settings[3,4]. However, long term effectiveness across different variants is still unknown, though reports of waning immunity are beginning to surface, not merely in terms of antibody dynamics over time[5–7], but in real-world settings as well[8].

Alongside the question of long-term protection provided by the vaccine, the degree and duration to which previous infection with SARS-CoV-2 affords protection against repeated infection also remains unclear. Apart from the paucity of studies examining long-term protection against reinfection[9], there is a challenge in defining reinfection as opposed to prolonged viral shedding[10]. While clear-cut cases exist, namely two separate clinical events with two distinct sequenced viruses, relying solely on these cases will likely result in an under-estimation of the incidence of reinfection. Different criteria based on more widely-available information have been suggested[11], the Centers for Disease Control and Prevention's (CDC) guidelines refer to two positive SARS-CoV-2 polymerase chain reaction (PCR) test results at least 90 days

apart.[12] Using similar criteria, population-based studies demonstrated natural immunity[13,14] with no signs of waning immunity for at least 7 months, though protection was lower for those aged 65 or older[9].

The Delta (B.1.617.2) Variant of Concern (VOC), initially identified in India and today globally prevalent, has been the dominant strain in Israel since June 2021. The recent surge of cases in Israel[15], one of the first countries to embark on a nationwide vaccination campaign (mostly with the BioNTech/Pfizer BNT162b2 vaccine), has raised concerns about vaccine effectiveness against the Delta variant, including official reports of decreased protection[16]. Concomitantly, studies have demonstrated only mild differences in short-term vaccine effectiveness[17] against the Delta variant, as well as substantial antibody response[18]. Apart from the variant, the new surge was also explained by the correlation found between time-from-vaccine and breakthrough infection rates, as early vaccinees were demonstrated to be significantly more at risk than late vaccinees[8]. Now, when sufficient time has passed since both the beginning of the pandemic and the deployment of the vaccine, we can examine the long-term protection of natural immunity compared to vaccine-induced immunity.

To this end, we compared the incidence rates of breakthrough infections to the incidence rates of reinfection, leveraging the centralized computerized database of Maccabi Healthcare Services (MHS), Israel's second largest Health Maintenance Organization.

Methods

Study design and population

A retrospective cohort study was conducted, leveraging data from MHS' centralized computerized database. The study population included MHS members aged 16 or older who were vaccinated prior to February 28, 2021, who had a documented SARS-CoV-2 infection by February 28, 2021, or who had both a documented SARS-CoV-2 infection by February 28, 2021 *and* received one dose of the vaccine by May 25, 2021, at least 7 days before the study period. On March 2, 2021, The Israeli Ministry of Health revised its guidelines and allowed previously SARS-CoV-2 infected individuals to receive one dose of the vaccine, after a minimum 3-month-interval from the date of infection

Data Sources

Anonymized Electronic Medical Records (EMRs) were retrieved from MHS' centralized computerized database for the study period of March 1, 2020 to August 14, 2021.

MHS is a 2.5-million-member, state-mandated, non-for-profit, second largest health fund in Israel, which covers 26% of the population and provides a representative sample of the Israeli population. Membership in one of the four national health funds is mandatory, whereas all citizens must freely choose one of four funds, which are prohibited by law from denying membership to any resident. MHS has maintained a centralized database of EMRs for three decades, with less than 1% disengagement rate among its members, allowing for a comprehensive longitudinal medical follow-up. The centralized dataset includes extensive demographic data, clinical measurements, outpatient and hospital diagnoses and procedures, medications

dispensed, imaging performed and comprehensive laboratory data from a single central laboratory.

Data extraction and definition of the study variables

COVID-19-related data

COVID-19-related information was captured as well, including dates of the first and second dose of the vaccine and results of any polymerase chain reaction (PCR) tests for SARS-CoV-2, given that all such tests are recorded centrally. Records of COVID-19-related hospitalizations were retrieved as well, and COVID-19-related mortality was screened for. Additionally, information about COVID-19-related symptoms was extracted from EMRs, where they were recorded by the primary care physician or a certified nurse who conducted in-person or phone visits with each infected individual.

Exposure variable: study groups

The eligible study population was divided into three groups: (1)fully vaccinated and SARS-CoV-2-naïve individuals, namely MHS members who received two doses of the BioNTech/Pfizer mRNA BNT162b2 vaccine by February 28, 2021, did not receive the third dose by the end of the study period and did not have a positive PCR test result by June 1, 2021; (2) unvaccinated previously infected individuals, namely MHS members who had a positive SARS-CoV-2 PCR test recorded by February 28, 2021 and who had not been vaccinated by the end of the study period; (3) previously infected *and* vaccinated individuals, including individuals who had a positive SARS-CoV-2 PCR test by February 28, 2021 and received one dose of the vaccine by May 25, 2021, at least 7 days before the study period. The fully vaccinated group was the comparison (reference) group in our study. Groups 2 and 3, were matched to the

comparison group 1 in a 1:1 ratio based on age, sex and residential socioeconomic status.

Dependent variables

We evaluated four SARS-CoV-2-related outcomes, or second events: documented RT-PCR confirmed SARS-CoV-2 infection, COVID-19, COVID-19-related hospitalization and death. Outcomes were evaluated during the follow-up period of June 1 to August 14, 2021, the date of analysis, corresponding to the time in which the Delta variant became dominant in Israel.

Covariates

Individual-level data of the study population included patient demographics, namely age, sex, socioeconomic status (SES) and a coded geographical statistical area (GSA, assigned by Israel's National Bureau of Statistics, corresponds to neighborhoods and is the smallest geostatistical unit of the Israeli census). The SES is measured on a scale from 1 (lowest) to 10, and the index is based on several parameters, including household income, educational qualifications, household crowding and car ownership. Data were also collected on last documented body mass index (BMI) and information about chronic diseases from MHS' automated registries, including cardiovascular diseases[19], hypertension[20], diabetes[21], chronic kidney disease[22], chronic obstructive pulmonary disease, immunocompromised conditions, and cancer from the National Cancer Registry[23].

Statistical analysis

Two multivariate logistic regression models were applied that evaluated the four aforementioned SARS-CoV-2-related outcomes as dependent variables, while the study groups were the main independent variables.

Model 1– previously infected vs. vaccinated individuals, with matching for time of first event

In model 1, we examined natural immunity and vaccine-induced immunity by comparing the likelihood of SARS-CoV-2-related outcomes between previously infected individuals who have never been vaccinated and fully vaccinated SARS-CoV-2-naïve individuals. These groups were matched in a 1:1 ratio by age, sex, GSA and time of first event. The first event (the preliminary exposure) was either the time of administration of the second dose of the vaccine *or* the time of documented infection with SARS-CoV-2 (a positive RT-PCR test result), both occurring between January 1, 2021 and February 28, 2021. Thereby, we matched the "immune activation" time of both groups, examining the long-term protection conferred when vaccination or infection occurred within the same time period. The three-month interval between the first event and the second event was implemented in order to capture reinfections (as opposed to prolonged viral shedding) by following the 90-day guideline of the CDC.

Model 2

In model 2, we compared the SARS-CoV-2 naïve vaccinees to unvaccinated previously infected individuals while intentionally *not* matching the time of the first event (i.e., either vaccination or infection), in order to compare vaccine-induced immunity to natural immunity, regardless of time of infection. Therefore, matching

was done in a 1:1 ratio based on age, sex and GSA alone. Similar to the model 1, either event (vaccination or infection) had to occur by February 28, to allow for the 90-day interval. The four SARS-CoV-2 study outcomes were the same for this model, evaluated during the same follow-up period.

Model 3

Model 3 examined previously infected individuals vs. previously-infected-and-once-vaccinated individuals, using "natural immunity" as the baseline group. We matched the groups in a 1:1 ratio based on age, sex and GSA. SARS-CoV-2 outcomes were the same, evaluated during the same follow-up period.

In all three models, we estimated natural immunity vs. vaccine-induced immunity for each SARS-CoV-2-related outcome, by applying logistic regression to calculate the odds ratio (OR) between the two groups in each model, with associated 95% confidence intervals (CIs). Results were then adjusted for underlying comorbidities, including obesity, cardiovascular diseases, diabetes, hypertension, chronic kidney disease, cancer and immunosuppression conditions.

Analyses were performed using Python version 3.73 with the stats model package. $P < 0.05$ was considered statistically significant.

Ethics declaration

This study was approved by the MHS (Maccabi Healthcare Services) Institutional Review Board (IRB). Due to the retrospective design of the study, informed consent was waived by the IRB, and all identifying details of the participants were removed before computational analysis.

Data availability statement

According to the Israel Ministry of Health regulations, individual-level data cannot be shared openly. Specific requests for remote access to de-identified community-level data should be directed to KSM, Maccabi Healthcare Services Research and Innovation Center.

Code availability

Specific requests for remote access to the code used for data analysis should be referred to KSM, Maccabi Healthcare Services Research and Innovation Center.

Results

Overall, 673,676 MHS members 16 years and older were eligible for the study group of fully vaccinated SARS-CoV-2-naïve individuals; 62,883 were eligible for the study group of unvaccinated previously infected individuals and 42,099 individuals were eligible for the study group of previously infected and single-dose vaccinees.

Model 1 – previously infected vs. vaccinated individuals, with matching for time of first event

In model 1, we matched 16,215 persons in each group. Overall, demographic characteristics were similar between the groups, with some differences in their comorbidity profile (Table 1a).

During the follow-up period, 257 cases of SARS-CoV-2 infection were recorded, of which 238 occurred in the vaccinated group (breakthrough infections) and 19 in the previously infected group (reinfections). After adjusting for comorbidities, we found a statistically significant 13.06-fold (95% CI, 8.08 to 21.11) increased risk for breakthrough infection as opposed to reinfection ($P<0.001$). Apart from age ≥60 years, there was no statistical evidence that any of the assessed comorbidities significantly affected the risk of an infection during the follow-up period (Table 2a).

As for symptomatic SARS-COV-2 infections during the follow-up period, 199 cases were recorded, 191 of which were in the vaccinated group and 8 in the previously infected group. Symptoms for all analyses were recorded in the central database within 5 days of the positive RT-PCR test for 90% of the patients, and included chiefly fever, cough, breathing difficulties, diarrhea, loss of taste or smell, myalgia, weakness, headache and sore throat. After adjusting for comorbidities, we found a 27.02-fold risk (95% CI, 12.7 to 57.5) for symptomatic breakthrough infection as

opposed to symptomatic reinfection ($P<0.001$) (Table 2b). None of the covariates were significant, except for age ≥60 years.

Nine cases of COVID-19-related hospitalizations were recorded, 8 of which were in the vaccinated group and 1 in the previously infected group (Table S1). No COVID-19-related deaths were recorded in our cohorts.

Model 2 –previously infected vs. vaccinated individuals, without matching for time of first event

In model 2, we matched 46,035 persons in each of the groups (previously infected vs. vaccinated). Baseline characteristics of the groups are presented in Table 1a. Figure 1 demonstrates the timely distribution of the first infection in reinfected individuals.

When comparing the vaccinated individuals to those previously infected at any time (including during 2020), we found that throughout the follow-up period, 748 cases of SARS-CoV-2 infection were recorded, 640 of which were in the vaccinated group (breakthrough infections) and 108 in the previously infected group (reinfections). After adjusting for comorbidities, a 5.96-fold increased risk (95% CI, 4.85 to 7.33) increased risk for breakthrough infection as opposed to reinfection could be observed ($P<0.001$) (Table 3a). Apart from SES level and age ≥60, that remained significant in this model as well, there was no statistical evidence that any of the comorbidities significantly affected the risk of an infection.

Overall, 552 symptomatic cases of SARS-CoV-2 were recorded, 484 in the vaccinated group and 68 in the previously infected group. There was a 7.13-fold (95% CI, 5.51 to 9.21) increased risk for symptomatic breakthrough infection than symptomatic reinfection (Table 3b). COVID-19 related hospitalizations occurred in 4 and 21 of the reinfection and breakthrough infection groups, respectively. Vaccinated

individuals had a 6.7-fold (95% CI, 1.99 to 22.56) increased to be admitted compared to recovered individuals. Being 60 years of age or older significantly increased the risk of COVID-19-related hospitalizations (Table S2). No COVID-19-related deaths were recorded.

Model 3 - previously infected vs. vaccinated and previously infected individuals

In model 3, we matched 14,029 persons. Baseline characteristics of the groups are presented in Table 1b. Examining previously infected individuals to those who were both previously infected and received a single dose of the vaccine, we found that the latter group had a significant 0.53-fold (95% CI, 0.3 to 0.92) (Table 4a) decreased risk for reinfection, as 20 had a positive RT-PCR test, compared to 37 in the previously infected and unvaccinated group. Symptomatic disease was present in 16 single dose vaccinees and in 23 of their unvaccinated counterparts. One COVID-19-related hospitalization occurred in the unvaccinated previously infected group. No COVID-19-related mortality was recorded.

We conducted a further sub-analysis, compelling the single-dose vaccine to be administered *after* the positive RT-PCR test. This subset represented 81% of the previously-infected-and-vaccinated study group. When performing this analysis, we found a similar, though not significant, trend of decreased risk of reinfection, with an OR of 0.68 (95% CI, 0.38 to 1.21, *P*-value=0.188).

Discussion

This is the largest real-world observational study comparing natural immunity, gained through previous SARS-CoV-2 infection, to vaccine-induced immunity, afforded by the BNT162b2 mRNA vaccine. Our large cohort, enabled by Israel's rapid rollout of the mass-vaccination campaign, allowed us to investigate the risk for additional infection – either a breakthrough infection in vaccinated individuals or reinfection in previously infected ones – over a longer period than thus far described.

Our analysis demonstrates that SARS-CoV-2-naïve vaccinees had a 13.06-fold increased risk for breakthrough infection with the Delta variant compared to those previously infected, when the first event (infection or vaccination) occurred during January and February of 2021. The increased risk was significant for a symptomatic disease as well.

Broadening the research question to examine the extent of the phenomenon, we allowed the infection to occur at any time between March 2020 to February 2021 (when different variants were dominant in Israel), compared to vaccination only in January and February 2021. Although the results could suggest waning natural immunity against the Delta variant, those vaccinated are still at a 5.96-fold increased risk for breakthrough infection and at a 7.13-fold increased risk for symptomatic disease compared to those previously infected. SARS-CoV-2-naïve vaccinees were also at a greater risk for COVID-19-related-hospitalization compared to those who were previously infected.

Individuals who were previously infected with SARS-CoV-2 seem to gain additional protection from a subsequent single-dose vaccine regimen. Though this finding corresponds to previous reports[24,25], we could not demonstrate significance in our cohort.

The advantageous protection afforded by natural immunity that this analysis demonstrates could be explained by the more extensive immune response to the SARS-CoV-2 proteins than that generated by the anti-spike protein immune activation conferred by the vaccine[26,27]. However, as a correlate of protection is yet to be proven[1,28], including the role of B-Cell[29] and T-cell immunity[30,31], this remains a hypothesis.

Our study has several limitations. First, as the Delta variant was the dominant strain in Israel during the outcome period, the decreased long-term protection of the vaccine compared to that afforded by previous infection cannot be ascertained against other strains. Second, our analysis addressed protection afforded solely by the BioNTech/Pfizer mRNA BNT162b2 vaccine, and therefore does not address other vaccines or long-term protection following a third dose, of which the deployment is underway in Israel. Additionally, as this is an observational real-world study, where PCR screening was not performed by protocol, we might be underestimating asymptomatic infections, as these individuals often do not get tested.

Lastly, although we controlled for age, sex, and region of residence, our results might be affected by differences between the groups in terms of health behaviors (such as social distancing and mask wearing), a possible confounder that was not assessed. As individuals with chronic illness were primarily vaccinated between December and February, confounding by indication needs to be considered; however, adjusting for obesity, cardiovascular disease, diabetes, hypertension, chronic kidney disease, chronic obstructive pulmonary disease, cancer and immunosuppression had only a small impact on the estimate of effect as compared to the unadjusted OR. Therefore, residual confounding by unmeasured factors is unlikely.

This analysis demonstrated that natural immunity affords longer lasting and stronger protection against infection, symptomatic disease and hospitalization due to the Delta variant of SARS-CoV-2, compared to the BNT162b2 two-dose vaccine-induced immunity. Notably, individuals who were previously infected with SARS-CoV-2 and given a single dose of the BNT162b2 vaccine gained additional protection against the Delta variant. The long-term protection provided by a third dose, recently administered in Israel, is still unknown.

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Tables and figures

Table 1a. Characteristics of study population, model 1 and 2.

	Model 1 – with matching of time of first event		**Model 2 – without matching of time of first event**	
Characteristics	Previously infected (n=16,215)	Vaccinated individuals (n=16,215)	Previously infected (n=46,035)	Previously infected *and* vaccinated (n =46,035)
Age years, mean (SD)	36.1 (13.9)	36.1 (13.9)	36.1 (14.7)	36.1 (14.7)
Age group – no. (%)				
16 to 39 yr	9,889 (61.0)	9,889 (61.0)	28,157 (61.2)	28,157 (61.2)
40 to 59 yr	5,536 (34.1)	5,536 (34.1)	14,973 (32.5)	14,973 (32.5)
≥60 yr	790 (4.9)	790 (4.9)	2,905 (6.3)	2,905 (6.3)
Sex – no. (%)				
Female	7,428 (45.8)	7,428 (45.8)	22,661 (49.2)	22,661 (49.2)
Male	8,787 (54.2)	8,787 (54.2)	23,374 (50.8)	23,374 (50.8)
SES, mean (SD)	5.5 (1.9)	5.5 (1.9)	5.3 (1.9)	5.3 (1.9)
Comorbidities – no. (%)				
Hypertension	1,276 (7.9)	1,569 (9.7)	4,009 (8.7)	4,301 (9.3)
CVD	551 (3.4)	647 (4.0)	1,875 (4.1)	1830 (4.0)
DM	635 (3.9)	877 (5.4)	2207 (4.8)	2300 (5.0)
Immunocompromised	164 (1.0)	420 (2.6)	527 (1.1)	849 (1.8)
Obesity (BMI ≥30)	3,076 (19.0)	3,073 (19.0)	9,117 (19.8)	8,610 (18.7)
CKD	196 (1.2)	271 (1.7)	659 (1.4)	814 (1.8)
COPD	65 (0.4)	97 (0.6)	218 (0.5)	292 (0.6)
Cancer	324 (2.0)	636 (3.9)	1,044 (2.3)	1,364 (3.0)

SD – Standard Deviation; SES – Socioeconomic status on a scale from 1 (lowest) to 10; CVD – Cardiovascular Diseases; DM – Diabetes Mellitus; CKD – Chronic Kidney Disease; COPD – Chronic Obstructive Pulmonary Disease.

Table 1b. Characteristics of study population, model 3.

Characteristics	Previously infected (n=14,029)	Previously infected and single dose vaccinated (n=14,029)
Age years, mean (SD)	33.2 (14.0)	33.2 (14.0)
Age group – no. (%)		
16 to 39 yr	9543 (68.0)	9543 (68.0)
40 to 59 yr	3919 (27.9)	3919 (27.9)
≥60 yr	567 (4.0)	567 (4.0)
Sex – no. (%)		
Female	7467 (53.2)	7467 (53.2)
Male	6562 (46.8)	6562 (46.8)
SES, mean (SD)	4.7 (1.9)	4.7 (1.9)
Comorbidities		
Hypertension	892 (6.4)	1004 (7.2)
CVD	437 (3.1)	386 (2.8)
DM	529 (3.8)	600 (4.3)
Immunocompromised	127 (0.9)	145 (1.0)
Obesity (BMI ≥30)	2599 (18.5)	2772 (19.8)
CKD	137 (1.0)	162 (1.2)
COPD	30 (0.2)	53 (0.4)
Cancer	241 (1.7)	267 (1.9)

SD – Standard Deviation; SES – Socioeconomic status on a scale from 1 (lowest) to 10; CVD – Cardiovascular Diseases; DM – Diabetes Mellitus; CKD – Chronic Kidney Disease; COPD – Chronic Obstructive Pulmonary Disease.

Table 2a. OR for SARS-CoV-2 infection, model 1, previously infected vs. vaccinated

Variable	Category	ß	OR	95%CI	P-value
Induced Immunity					
	Previously infected	Ref			
	Vaccinated	2.57	13.06	8.08 – 21.11	<0.001
SES		0.04	1.04	0.97 – 1.11	0.251
Age group, yr.					
	16-39	Ref			
	40-59	0.05	1.05	0.78 - 1.4	0.751
	≥60	0.99	2.7	1.68 – 4.34	<0.001
Sex					
	Female	Ref			
	Male	-0.03	0.97	0.76 – 1.25	0.841
Comorbidities					
	Obesity (BMI≥30)	0.01	1.01	0.73 – 1.39	0.967
	Diabetes mellitus	-0.36	0.7	0.39 – 1.25	0.229
	Hypertension	0.1	1.11	0.72 – 1.72	0.641
	Cancer	0.37	1.44	0.85 – 2.44	0.171
	CKD	0.53	1.7	0.83 – 3.46	0.146
	COPD	-0.46	0.63	0.15 – 2.66	0.529
	Immunosuppression	-0.1	0.91	0.42 – 1.97	0.803
	Cardiovascular diseases	0.26	1.3	0.75 – 2.25	0.343

OR – Odds Ratio; SES – Socioeconomic status on a scale from 1 (lowest) to 10; CVD – Cardiovascular Diseases; CKD – Chronic Kidney Disease; COPD – Chronic Obstructive Pulmonary Disease.

Table 2b. OR for Symptomatic SARS-CoV-2 infection, model 1, previously infected vs. vaccinated

Variable	Category	ß	OR	95%CI	P-value
Induced Immunity					
	Previously infected	Ref			
	Vaccinated	3.3	27.02	12.7 – 57.5	<0.001
SES		0.04	1.04	0.96 – 1.12	0.312
Age group, yr.					
	16-39	Ref			
	40-59	0.19	1.21	0.88 – 1.67	0.25
	≥60	1.06	2.89	1.68 – 4.99	<0.001
Sex					
	Female	Ref			
	Male	-0.19	0.82	0.62 – 1.1	0.185
Comorbidities					
	Obesity (BMI≥30)	0.02	1.02	0.71 – 1.48	0.899
	Diabetes mellitus	-0.31	0.73	0.37 – 1.43	0.361
	Hypertension	0.12	1.13	0.69 – 1.85	0.623
	Cancer	0.37	1.45	0.8 – 2.62	0.217
	CKD	0.1	1.1	0.42 – 2.87	0.846
	COPD	-0.78	0.46	0.06 – 3.41	0.445
	Immunosuppression	-0.37	0.69	0.25 – 1.89	0.468
	Cardiovascular diseases	0.03	1.03	0.52 – 2.03	0.941

OR – Odds Ratio; SES – Socioeconomic status on a scale from 1 (lowest) to 10; CVD – Cardiovascular Diseases; CKD – Chronic Kidney Disease; COPD – Chronic Obstructive Pulmonary Disease.

Table 3a. OR for SARS-CoV-2 infection, model 2, previously infected vs. vaccinated

Variable	Category	ß	OR	95%CI	P-value
Induced Immunity					
	Previously infected	Ref			
	Vaccinated	1.78	5.96	4.85 – 7.33	<0.001
SES		0.07	1.07	1.03 – 1.11	<0.001
Age group, yr.					
	16-39	Ref			
	40-59	0.06	1.06	0.9 – 1.26	0.481
	≥60	0.79	2.2	1.66 – 2.92	<0.001
Sex					
	Female	Ref			
	Male	-0.01	0.99	0.85 - 1.14	0.842
Comorbidities					
	Obesity (BMI≥30)	0.12	1.13	0.94 – 1.36	0.202
	Diabetes mellitus	-0.15	0.86	0.61 – 1.22	0.4
	Hypertension	-0.12	0.89	0.67 – 1.17	0.402
	Cancer	0.2	1.22	0.85 – 1.76	0.283
	CKD	0.3	1.35	0.85 – 2.14	0.207
	COPD	0.48	1.62	0.88 – 2.97	0.121
	Immunosuppression	-0.03	0.98	0.57 – 1.66	0.925
	Cardiovascular diseases	0.08	1.09	0.77 – 1.53	0.638

OR – Odds Ratio; SES – Socioeconomic status on a scale from 1 (lowest) to 10; CVD – Cardiovascular Diseases; CKD – Chronic Kidney Disease; COPD – Chronic Obstructive Pulmonary Disease.

Table 3b. OR for Symptomatic SARS-CoV-2 infection, model 2, previously infected vs. vaccinated

Variable	Category	ß	OR	95%CI	P-value
Induced Immunity					
	Previously infected	Ref			
	Vaccinated	1.96	7.13	5.51 – 9.21	<0.001
SES		0.07	1.07	1.02 – 1.12	0.003
Age group, yr.					
	16-39	Ref			
	40-59	0.09	1.1	0.9 – 1.33	0.35
	≥60	0.8	2.23	1.61 – 3.09	<0.001
Sex					
	Female	Ref			
	Male	-0.02	0.98	0.82 – 1.16	0.785
Comorbidities					
	Obesity (BMI≥30)	0.16	1.18	0.95 – 1.46	0.133
	Diabetes mellitus	-0.11	0.89	0.61 – 1.32	0.571
	Hypertension	-0.01	0.99	0.72 – 1.35	0.943
	Cancer	0.08	1.09	0.7 – 1.69	0.71
	CKD	0.13	1.14	0.65 – 1.98	0.654
	COPD	0.5	1.65	0.82 – 3.31	0.162
	Immunosuppression	0	1	0.54 – 1.85	0.999
	Cardiovascular diseases	0	1	0.67 – 1.5	0.99

OR – Odds Ratio; SES – Socioeconomic status on a scale from 1 (lowest) to 10; CVD – Cardiovascular Diseases; CKD – Chronic Kidney Disease; COPD – Chronic Obstructive Pulmonary Disease.

Table 4a. OR for SARS-CoV-2 infection, model 3, previously infected vs. previously infected and single-dose-vaccinated

Variable	Category	ß	OR	95%CI	P-value
Induced Immunity					
	Previously infected	Ref			
	Previously infected and vaccinated	-0.64	0.53	0.3 – 0.92	0.024
SES		0.11	1.12	0.98 – 1.28	0.096
Age group, yr.					
	16-59	Ref			
	≥60	-0.81	0.44	0.06 – 3.22	0.422
Comorbidities					
	Immunosuppression	0.72	2.06	0.28 – 15.01	0.475

SES – Socioeconomic status on a scale from 1 (lowest) to 10

Table 4b. OR for Symptomatic SARS-CoV-2 infection, model 2, previously infected vs. previously infected and vaccinated

Variable	Category	ß	OR	95%CI	P-value
Induced Immunity					
	Previously infected	Ref			
	Previously infected and vaccinated	-0.43	0.65	0.34 – 1.25	0.194
SES		0.06	1.06	0.9 – 1.24	0.508
Age group, yr.					
	16-59	Ref			
	≥60	-16.9	0	0.0 – inf	0.996
Comorbidities					
	Immunosuppression	1.15	3.14	0.43 – 23.01	0.26

OR – Odds Ratio; SES – Socioeconomic status on a scale from 1 (lowest) to 10.

Table S1. OR for COVID-19-related hospitalizations, model 1, previously infected vs. vaccinated

Variable	Category	ß	OR hospitalized	95%CI	*P*-value
Induced Immunity					
	Previously infected	Ref			
	Vaccinated	2.09	8.06	1.01 – 64.55	0.049
SES		0.05	1.05	0.72 – 1.53	0.81
Age ≥60 yrs (16-39, ref)		5.08	160.9	19.91 – 1300.44	<0.001

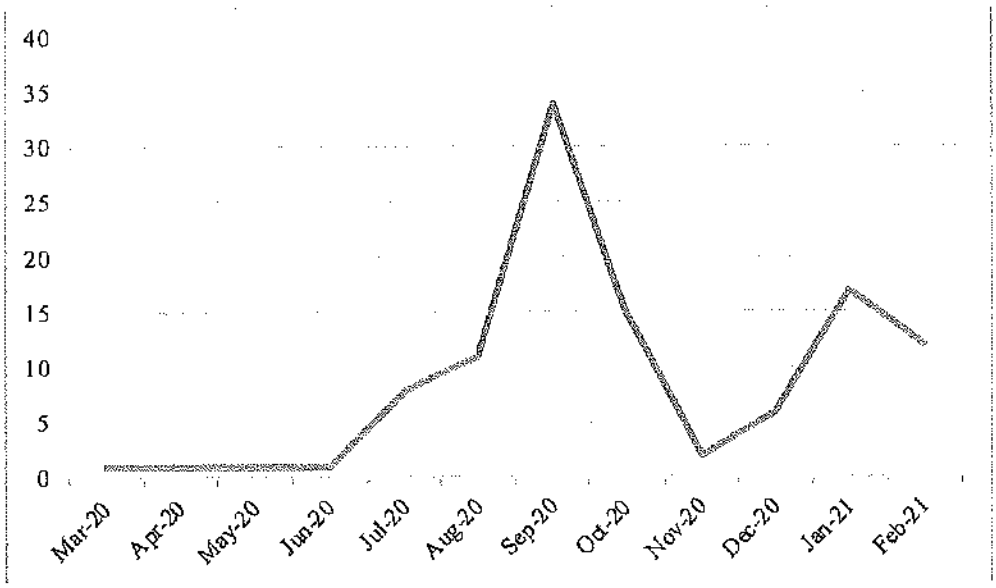
OR – Odds Ratio; SES – Socioeconomic status on a scale from 1 (lowest) to 10

Table S2. OR for COVID-19-related hospitalizations, model 2, previously infected vs. vaccinated

Variable	Category	ß	OR hospitalized	95%CI	*P*-value
Induced Immunity					
	Previously infected	Ref			
	Vaccinated	1.95	7.03	2.1 – 23.59	0.002
SES		-0.07	0.93	0.74 – 1.17	0.547
Age ≥60 yrs (16-39, ref)		4.3	73.5	25.09 – 215.29	<0.001

OR – Odds Ratio; SES – Socioeconomic status on a scale from 1 (lowest) to 10

Figure 1. Time of first infection in those reinfected between June and August 2021, model 2.



Necessity of COVID-19 vaccination in previously infected individuals

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Summary: Cumulative incidence of COVID-19 was examined among 52238 employees in an American healthcare system. COVID-19 did not occur in anyone over the five months of the study among 2579 individuals previously infected with COVID-19, including 1359 who did not take the vaccine.

ABSTRACT

Background. The purpose of this study was to evaluate the necessity of COVID-19 vaccination in persons previously infected with SARS-CoV-2.

Methods. Employees of the Cleveland Clinic Health System working in Ohio on Dec 16, 2020, the day COVID-19 vaccination was started, were included. Any subject who tested positive for SARS-CoV-2 at least 42 days earlier was considered previously infected. One was considered vaccinated 14 days after receipt of the second dose of a SARS-CoV-2 mRNA vaccine. The cumulative incidence of SARS-CoV-2 infection over the next five months, among previously infected subjects who received the vaccine, was compared with those of previously infected subjects who remained unvaccinated, previously uninfected subjects who received the vaccine, and previously uninfected subjects who remained unvaccinated.

Results. Among the 52238 included employees, 1359 (53%) of 2579 previously infected subjects remained unvaccinated, compared with 20804 (42%) of 49659 not previously infected. The cumulative incidence of SARS-CoV-2 infection remained almost zero among previously infected unvaccinated subjects, previously infected subjects who were vaccinated, and previously uninfected subjects who were vaccinated, compared with a steady increase in cumulative incidence among previously uninfected subjects who remained unvaccinated. Not one of the 1359 previously infected subjects who remained unvaccinated had a SARS-CoV-2 infection over the duration of the study. In a Cox proportional hazards regression model, after adjusting for the phase of the epidemic, vaccination was associated with a significantly lower risk of SARS-CoV-2 infection among those not previously infected (HR 0.031, 95% CI 0.015 to 0.061) but not among those previously infected (HR 0.313, 95% CI 0 to Infinity).

Conclusions. Individuals who have had SARS-CoV-2 infection are unlikely to benefit from COVID-19 vaccination, and vaccines can be safely prioritized to those who have not been infected before.

INTRODUCTION

The two FDA-approved (BNT162b2 mRNA [Pfizer-BioNTech] and mRNA-1273 [Moderna]) mRNA vaccines have been shown to be very efficacious in protecting against Severe Acute Respiratory Syndrome (SARS) – associated Coronavirus-2 (SARS-CoV-2) infection [1,2]. The effectiveness of the Pfizer-BioNTech vaccine in a real-world setting has also been shown to be comparable to the efficacy demonstrated in clinical trials [3,4]. Given these, there has been an understandable desire to vaccinate as many people as possible.

The ability to vaccinate a large part of the population is limited by the supply of vaccine. As of March 21, 2021, 78% of 447 million doses of the coronavirus disease 2019 (COVID-19) vaccines that had been deployed had gone to only ten countries [5]. The COVAX initiative was borne out of the recognition that equitable distribution of vaccines worldwide was essential for effective control of the COVID-19 pandemic. However, the reality is that there is great disparity in the availability of vaccines across countries. Countries with limited supplies of vaccine have to prioritize how their supply of vaccines will be allocated within their populations. Criteria used for such prioritization have included profession, age, and comorbid conditions. Data that inform prioritization criteria with help maximize the benefits of whatever vaccine is available.

Observational studies have found very low rates of reinfection among individuals with prior SARS-CoV-2 infection [6–8]. This brings up the question about whether it is necessary to vaccinate previously infected individuals. These studies notwithstanding, there remains a theoretical possibility that the vaccine may still provide some benefit in previously infected persons. A prior large observational study concluded that immunity from natural infection cannot be relied on to provide adequate protection and advocated for vaccination of previously infected individuals [9]. The CDC website recommends that persons previously infected with SARS-CoV-2 still get the vaccine [10]. Despite these recommendations, credible reports of previously infected persons getting COVID-19 are rare. The rationale often provided for getting the COVID-19 vaccine is that it is safer to get vaccinated than to get the disease. This is

certainly true, but it is not an explanation for why people who have already had the disease need to be vaccinated. A strong case for vaccinating previously infected persons can be made if it can be shown that previously infected persons who are vaccinated have a lower incidence of COVID-19 than previously infected persons who did not receive the vaccine.

The purpose of this study was to attempt to do just that, and thereby evaluate the necessity of the COVID-19 vaccine in persons who were previously infected with SARS-CoV-2.

METHODS

Study design

This was a retrospective cohort study conducted at the Cleveland Clinic Health System in Ohio, USA. The study was approved by the Cleveland Clinic Institutional Review Board. A waiver of informed consent and waiver of HIPAA authorization were approved to allow access to personal health information by the research team, with the understanding that sharing or releasing identifiable data to anyone other than the study team was not permitted without additional IRB approval.

Setting

PCR testing for SARS-CoV-2 at Cleveland Clinic began on March 12, 2020, and a streamlined process dedicated to the testing of health care personnel (HCP) was begun shortly thereafter. All employees with a positive SARS-CoV-2 test were interviewed by Occupational Health, with date of onset of symptoms of COVID-19 being one of the questions asked. Vaccination for COVID-19 began at Cleveland Clinic on December 16, 2020. When initially started it was the Pfizer-BioNTech vaccine that was administered, until the Moderna vaccine became available, from which time employees received one or the other. All employees were scheduled to receive their second vaccine dose 28 days after the first one, regardless of which vaccine was given. The employee cohort was chosen for this study because of documentation of their COVID-19 vaccination and of any SARS-CoV-2 infection in the Occupational Health database.

Participants

All employees of the Cleveland Clinic Health System, working in Ohio, on Dec 16, 2020, were screened for inclusion in the study. Those who were in employment on December 16, 2020, were included.

Variables

SARS-CoV-2 infection was defined as a positive nucleic acid amplification test. The date of infection was taken to be the date of onset of symptoms when available, and the date of specimen collection when not. A person was considered vaccinated 14 days after receipt of the second dose of the vaccine (which would have been 42 days after receipt of the first dose of the vaccine for most subjects). For the sake of consistency in the duration assumed for development of natural and vaccine immunity, any person who tested positive for SARS-CoV-2 at least 42 days before the vaccine rollout date, was considered previously infected. Other covariates collected were age, job location, job type (patient-facing or non-patient facing), and job category. The job location variable could be one of the following: Cleveland Clinic Main Campus, regional hospital (within Ohio), ambulatory center, administrative center, or remote location. The job category was one of the following: professional staff, residents/fellows, advance practice practitioners, nursing, pharmacy, clinical support, research, administration, and administration support.

Outcome

The study outcome was time to SARS-CoV-2 infection, the latter defined as a positive nucleic acid amplification test for SARS-CoV-2 on or after December 16, 2020. Time to SARS-CoV-2 infection was calculated as number of days from December 16, 2020 (vaccine rollout date) to SARS-CoV-2 infection. For those with a prior SARS-CoV-2 infection positive tests within 90 days of the first positive test were considered part of the initial episode of illness. Employees that had not developed a SARS-CoV-2 infection were censored at the end of the study follow-up period (May 15, 2021). Those who received the Johnson & Johnson vaccine (81 subjects) without having had a SARS-CoV-2 infection were censored on the day of receipt of the vaccine, and those whose employment was terminated during the study period before they had SARS-CoV-2 infection (2245 subjects) were censored on the date of

termination of employment. The health system never had a requirement for asymptomatic employee test screening. Most of the positive tests, therefore, would have been tests done to evaluate suspicious symptoms. A small proportion would have been tests done as part of pre-operative or pre-procedural screening.

Statistical analysis

A Simon-Makuch hazard plot [11] was created to compare the cumulative incidence of SARS-CoV-2 infection among previously infected subjects who were vaccinated, with those of previously infected subjects who remained unvaccinated, previously uninfected subjects who were vaccinated, and previously uninfected subjects who remained unvaccinated. Previous infection was treated as a time-independent covariate (SARS-CoV-2 infection at least 42 days before Dec 16, 2020), and vaccination (14 days after receipt of the second dose of the vaccine) was treated as a time-dependent covariate (Figure 1). Curves for the unvaccinated were based on data for those who did not receive the vaccine over the duration of the study, and for those who did until the date they were considered vaccinated, from which point onwards their data were recorded into the corresponding vaccinated set. A Cox proportional hazards regression model was fitted with time to SARS-CoV-2 infection as the outcome variable against vaccination (as a time-dependent covariate whose value changed on the date a subject was considered vaccinated)[12]. Previous infection (as a time-independent covariate) and an interaction term for previous infection and vaccination were included as covariates. The phase of the epidemic was adjusted for by including the slope of the epidemic curve as a time-dependent covariate whose value changed continuously with the slope of the epidemic curve. The analysis was performed by NKS and ASN using the *survival* package and R version 4.0.5 [12–14].

RESULTS

Of 52238 employees included in the study, 2579 (5%) were previously infected with SARS-CoV-2.

Baseline characteristics

Those previously infected with SARS-CoV-2 were significantly younger (mean ± SD age; 39 ± 13 vs. 42 ± 13, p<0.001), and included a significantly higher proportion with patient-facing jobs (65% vs. 51%, p<0.001). Table 1 shows the characteristics of subjects grouped by whether or not they were previously infected. A significantly lower proportion of those previously infected (47%, 1220 subjects) were vaccinated by the end of the study compared to 58% (28855) of those not previously infected (p<0.001). Of those vaccinated, 63% received the Moderna vaccine. Twelve percent of subjects with previous SARS-CoV-2 infection did not have a symptom onset date, suggesting they may possibly have been identified on pre-operative or pre-procedural screening, and may not have had symptomatic infection. When vaccination was begun, the epidemic in Ohio was at the peak of its third wave (Figure 2).

Cumulative Incidence of COVID-19

Figure 3 is a Simon-Makuch plot showing that SARS-CoV-2 infections occurred almost exclusively in subjects who were not previously infected with SARS-CoV-2 and who remained unvaccinated. The cumulative incidence of SARS-CoV-2 infection among previously infected unvaccinated subjects did not differ from that of previously infected subjects who were vaccinated, and that of previously uninfected subjects who were vaccinated. For all three of these groups, the cumulative incidence of SARS-CoV-2 infection was much lower than that of subjects who were not previously infected and who remained unvaccinated. Of the 2154 SARS-CoV-2 infections during the study period, 2139 (99.3%) occurred among those not previously infected who remained unvaccinated or were waiting

to get vaccinated, and15 (0.7%) occurred among those not previously infected who were vaccinated. Not one of the 2579 previously infected subjects had a SARS-CoV-2 infection, including 1359 who remained unvaccinated throughout the duration of the study.

Association of vaccination with occurrence of COVID-19

In a Cox proportional hazards regression model, after adjusting for the phase of the epidemic, vaccination was associated with a significantly lower risk of SARS-CoV-2 infection among those not previously infected (HR 0.031, 95% CI 0.015 – 0.061) but not among those previously infected (HR 0.313, 95% CI 0 – Infinity). The absence of events among those who were previously infected, whether they received the vaccine or not, precluded accurate or precise estimates for the latter effect size.

Duration of protection

This study was not specifically designed to determine the duration of protection afforded by natural infection, but for the previously infected subjects the median duration since prior infection was 143 days (IQR 76 – 179 days), and no one had SARS-CoV-2 infection over the following five months, suggesting that SARS-CoV-2 infection may provide protection against reinfection for 10 months or longer.

DISCUSSION

This study shows that subjects previously infected with SARS-CoV-2 are unlikely to get COVID-19 reinfection whether or not they receive the vaccine. This finding calls into question the necessity to vaccinate those who have already had SARS-CoV-2 infection.

It is reasonable to expect that immunity acquired by natural infection provides effective protection against future infection with SARS-CoV-2. Observational studies have indeed found very low rates of reinfection over the following months among survivors of COVID-19 [6–8]. Reports of true reinfections are extremely rare in the absence of emergence of new variants. When such reinfections occur, it would be purely speculative to suggest that a vaccine might have prevented them. Duration of protective immunity from natural infection is not known. However, the same also can be said about duration of protective immunity from vaccination. Uncertainty about the duration of protective immunity afforded by natural infection is not by itself a valid argument for vaccinating previously infected individuals. This study provides direct evidence that vaccination with the best available vaccines does not provide additional protection in previously infected individuals.

A prior study concluded that natural infection cannot be relied on to protect against COVID-19 [9]. That study was based on comparison of PCR-positivity rates during a second COVID-19 surge in Denmark between those who tested positive and negative during the first COVID-19 surge, and indirectly calculated that prior infection provided 80.5% protection against repeat infection, and that protection against those older than 65 years was only 47.1%. The study did not compare vaccinated and unvaccinated people, and it is therefore an assumption to consider that a vaccine would have provided better protection in that particular population. Furthermore, there was a gap of only seven weeks between the end of the first surge and the beginning of the second in that study. It is now well-known that a small number of people can continue to have positive PCR test results for several weeks to a few months after infection, one study finding that 5.3% remained positive at 90 days [15]. It is possible that some of the positives picked up in the early part of the second surge were not necessarily new infections but residual

virus from the tail end of the first surge. Since the actual number of infections was small, a few such misclassifications could change the rates substantially. Our study examined rates of SARS-CoV-2 infection in vaccinated and unvaccinated individuals and showed that those previously infected who did not receive the vaccine did not have higher rates of SARS-CoV-2 infection than those previously infected who did, thereby providing direct evidence that vaccination does not add protection to those who were previously infected.

There are several strengths to our study. Its large sample size and follow-up of up to 5 months provide us with an ample degree of confidence in its findings. A major strength of our study is that we adjusted the analyses for the phase of the epidemic at all time points. The risk of acquisition of infection is strongly influenced by the phase of the epidemic at any given time, and it is important to adjust for this for accurate risk analyses. Given that was this a study among employees of a health system, and that the health system had policies and procedures in recognition of the critical importance of keeping track of the pandemic among its employees, we had an accurate accounting of who had COVID-19, when they were diagnosed with COVID-19, who received a COVID-19 vaccine, and when they received it.

The study has its limitations. Because we did not have a policy of asymptomatic employee screening, previously infected subjects who remained asymptomatic might have been misclassified as previously uninfected. Given this limitation, one should be cautious about drawing conclusions about the protective effect of prior asymptomatic SARS-CoV-2 infection. It should be noted though, that 12% of the subjects classified as previously infected did not have a symptom onset date recorded, suggesting that at least some of those classified as previously infected might have been asymptomatic infections. It is reassuring that none of these possibly asymptomatically infected individuals developed COVID-19 during the duration of the study. The study follow-up duration was short, being only five months, but this was longer than published mRNA vaccine efficacy studies [1,2], and longer than the follow-up duration of the largest published vaccine effectiveness studies to date [3,4]. Median freedom from reinfection (time from initial infection until end of follow-up) in this study, for those previously infected, of almost 10 months, is consistent with findings in an earlier study that immunoglobulin G (IgG) to the spike protein remained

stable over more than six months after an episode of infection [16]. Our study included no children and few elderly subjects, and the majority would not have been immunosuppressed. Data governance policies in our institution precluded us from obtaining detailed clinical information on employees. While one cannot generalize this study's findings to assume that prior infection would provide adequate immunity in these groups, there is also no reason to expect a vaccine to provide additional protection in these same groups. Lastly, it is necessary to emphasize that these findings are based on the prevailing assortment of virus variants in the community during the study. It is not known how well these results will hold if or when some of the newer variants of concern become prominent. However, if prior infection does not afford protection against some of the newer variants of concern, there is little reason to suppose that the currently available vaccines would either. Vaccine breakthrough infections with variants have indeed been reported [17].

Our study's findings have important implications. Worldwide, COVID-19 vaccines are still in short supply. As of March 9, 2021, dozens of countries had not been able to administer a single dose of the vaccine [18]. As of May 17, 2021, only 17 countries had been able to reach ten percent or more of their populations with at least the first dose of vaccine [19]. Given such a scarcity of the vaccine, and the knowledge that vaccine does not provide additional protection to those previously infected, it would make most sense to limit vaccine administration to those who have not previously had the infection. In addition to profession, age, and comorbid conditions, previous infection should be an important consideration in deciding whom to prioritize to receive the vaccine. A practical and useful message would be to consider symptomatic COVID-19 to be as good as having received a vaccine, and that people who have had COVID-19 confirmed by a reliable laboratory test do not need the vaccine.

In conclusion, individuals who have laboratory-confirmed symptomatic SARS-CoV-2 infection are unlikely to benefit from COVID-19 vaccination, and vaccines can be safely prioritized to those who have not been infected before.

TRANSPARENCY DECLARATION

Conflict of Interest

Selection of "no competing interests" reflects that all authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

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Author contributions

NKS: Conceptualization, Methodology, Validation, Investigation, Data curation, Software, Formal analysis, Visualization, Writing- Original draft preparation, Writing- Reviewing and Editing, Supervision, Project administration.

ASN: Methodology, Formal analysis, Visualization, Validation, Writing- Reviewing and Editing.

PCB: Resources, Investigation, Validation, Writing- Reviewing and Editing.

PT: Resources, Writing- Reviewing and Editing.

SMG: Project administration, Resources, Writing- Reviewing and Editing.

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TABLES

Table 1. Study Subject Characteristics

Characteristic	Previously Infected (N = 2579)	Not Previously Infected (N = 49659)	*P* Value
Age, y, mean ± SD	39±13	42±13	<0.001
Patient-facing job	1676 (65)	25504 (51)	<0.001
Job location			<0.001
Cleveland Clinic Main Campus	1011 (39)	19595 (40)	
Regional hospitals	1096 (43)	16433 (33)	
Ambulatory centers	313 (12)	7767 (16)	
Administrative centers	138 (5)	4424 (9)	
Remote location	21 (<1)	1440 (3)	
Job category			<0.001
Professional staff	89 (4)	3775 (8)	
Residents and fellows	72 (3)	1669 (3)	
Advanced practice practitioners	154 (6)	2806 (6)	
Nursing	1142 (44)	13623 (27)	
Pharmacy	44 (2)	1274 (3)	
Research	328 (13)	6776 (14)	
Clinical support	111 (4)	3500 (7)	
Administration	614 (24)	15050(30)	
Administration support	25 (1)	1186 (2)	

Data are presented as no. (%) unless otherwise indicated

FIGURES

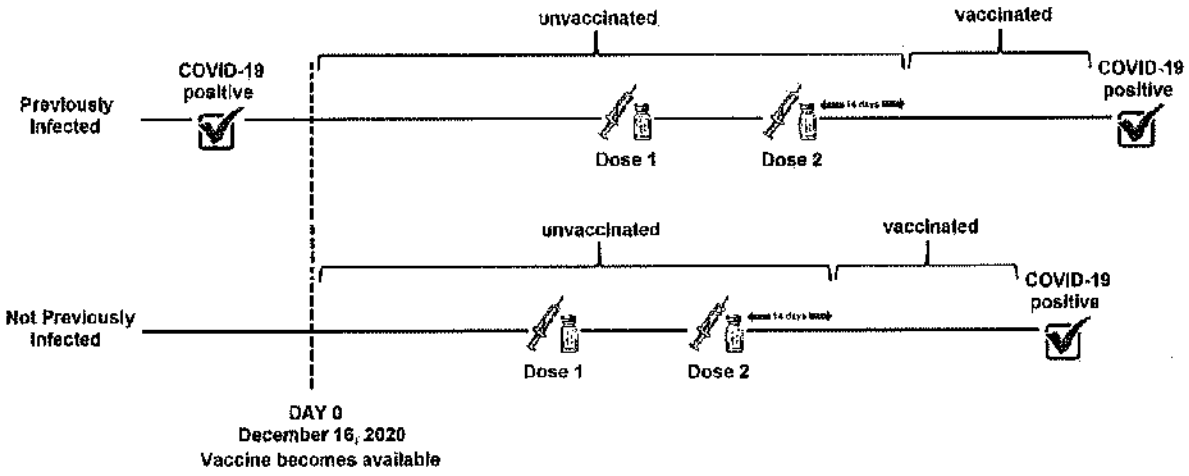


Figure 1. Explanation of "previously infected" analyzed as a time-independent covariate and "vaccinated" treated as a time-dependent covariate.

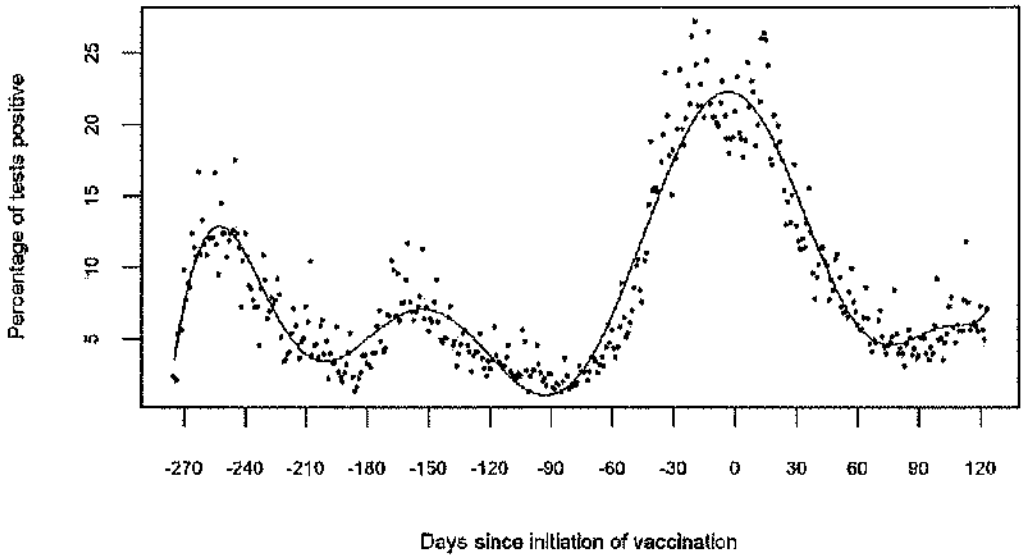
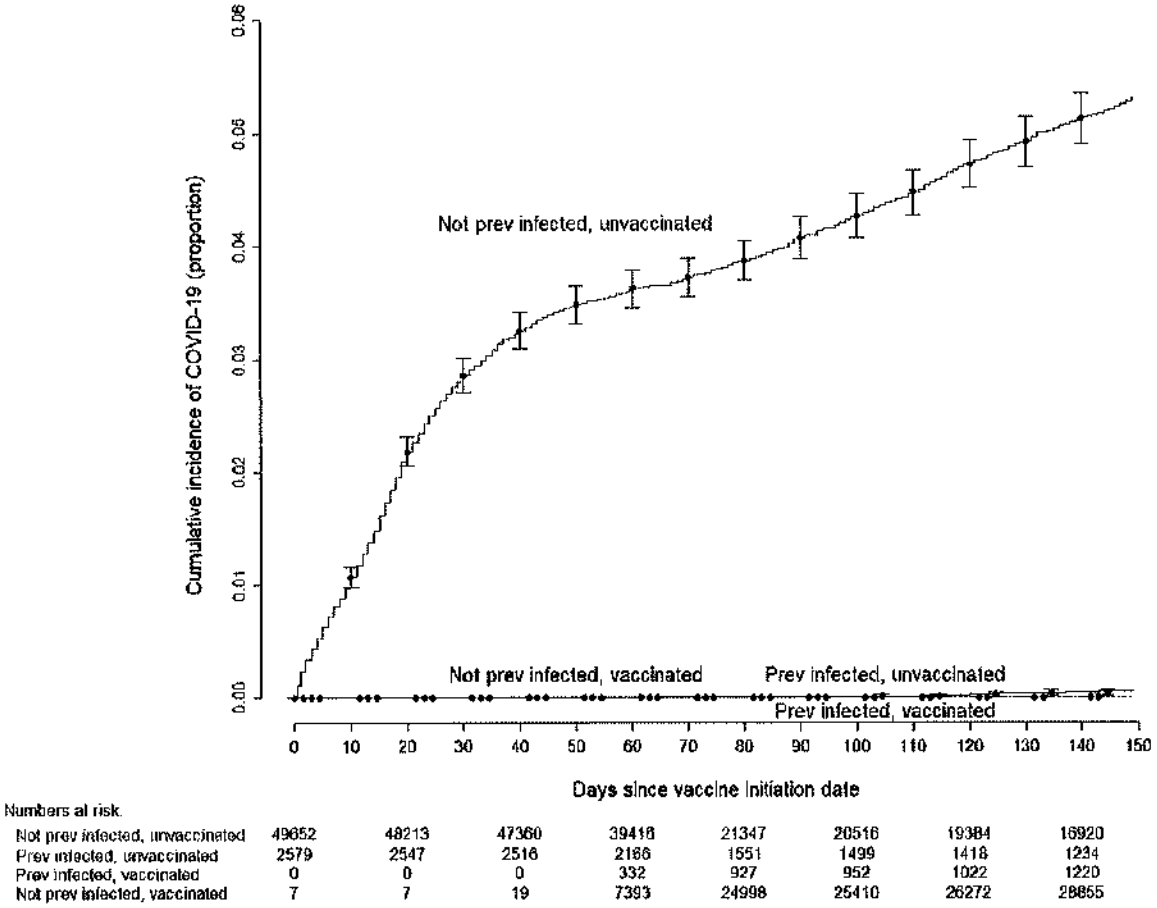


Figure 2. COVID-19 epidemic curve before and after vaccine rollout. Points on the scatter plot represent the proportion of all COVID-19 PCR tests done at Cleveland Clinic that were positive on any given day. The colored line represents a fitted polynomial curve.



Numbers at risk

Not prev infected, unvaccinated	49652	48213	47360	39418	21347	20516	19384	16920
Prev infected, unvaccinated	2579	2547	2516	2166	1551	1499	1418	1234
Prev infected, vaccinated	0	0	0	332	927	952	1022	1220
Not prev infected, vaccinated	7	7	19	7393	24998	25410	26272	28855

Figure 3. Simon-Makuch plot showing the cumulative incidence of COVID-19 among subjects previously infected and not previously infected with COVID-19, who did and did not receive the vaccine. Curves for the unvaccinated are based on data for those who did not receive the vaccine during the duration of the study, and for those waiting to receive the vaccine. Day zero was Dec 16, 2020, the day vaccination was started in our institution. Error bars represent 95% confidence intervals. Seven subjects who had been vaccinated earlier as participants in clinical trials were considered vaccinated throughout the duration of the study. Twelve subjects who received their first dose in the first week of the vaccination campaign managed to get their second dose three weeks later, and were thus considered vaccinated earlier than 42 days since the start of the vaccination campaign.

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European Journal of Immunology

Immunity to infection

Clinical

Research Article

Persistence of neutralizing antibodies a year after SARS-CoV-2 infection in humans

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Most subjects develop antibodies to SARS-CoV-2 following infection. In order to estimate the duration of immunity induced by SARS-CoV-2 it is important to understand for how long antibodies persist after infection in humans. Here, we assessed the persistence of serum antibodies following WT SARS-CoV-2 infection at 8 and 13 months after diagnosis in 367 individuals. The SARS-CoV-2 spike IgG (S-IgG) and nucleoprotein IgG (N-IgG) concentrations and the proportion of subjects with neutralizing antibodies (NAb) were assessed. Moreover, the NAb titers among a smaller subset of participants ($n = 78$) against a WT virus (B) and variants of concern (VOCs): Alpha (B.1.1.7), Beta (B.1.351), and Delta (B.1.617.2) were determined. We found that NAb against the WT virus persisted in 89% and S-IgG in 97% of subjects for at least 13 months after infection. Only 36% had N-IgG by 13 months. The mean S-IgG concentrations declined from 8 to 13 months by less than one third; N-IgG concentrations declined by two-thirds. Subjects with severe infection had markedly higher IgG and NAb levels and are expected to remain seropositive for longer. Significantly lower NAb titers against the variants compared to the WT virus, especially after a mild disease, suggests reduced protection against VOCs.

Keywords: IgG antibodies · neutralizing antibodies · SARS-CoV-2 · seroprevalence · variants of concern

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Introduction

Infection with Severe acute respiratory coronavirus 2 (SARS-CoV-2) induces antibodies in most subjects to viral nucleoprotein (N) and spike (S) glycoprotein (1). Neutralizing antibodies (NAb) against SARS-CoV-2 target the receptor-binding domain (RBD) of the S protein and sterically interfere with the binding of the viral S protein and the host's angiotensin-converting enzyme 2 (2, 3). NAb levels are highly predictive of protection against infection and clinical disease (4) and detectable NAb have been reported to persist in most subjects at least 6 to 12 months after infection (5–13). Previous findings suggest that neutralizing activity against the SARS-CoV-2 is mediated particularly by IgG1 and IgA antibodies (14, 15). However, as the concentration of anti-SARS-CoV-2 IgA antibodies has been shown to decline rapidly following

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[#]Both authors contributed equally to this work.

Table 1. Demographics and clinical characteristics of study participants in the study cohorts at 8 and 13 months after infection

	8 months participants	13 months participants	Study Cohort	Sub Cohort
N				
8 months	1292	N/A	367	N/A
13 months	N/A	995	367	78
Gender				
Male n (%)	520 (40%)	386 (39%)	159 (43%)	40 (51%)
Female n (%)	772 (60%)	609 (61%)	208 (57%)	38 (49%)
Age at diagnosis (median, range)				
<60y	45.1 (17.3-59.9)	47.5(17.6-59.9)	45.9 (17.7-59.9)	51.6 (19.0-59.7)
>60y	65.1 (60.0-94.3)	65.4 (60.0-95.6)	63.3 (60.0-79.0)	63.0 (60.0-81.3)
All	50.0 (17.3-94.3)	52.5 (17.6-95.6)	48.8 (17.7-79.0)	59.4 (19.0-81.3)
Time (mo) after diagnosis at sampling				
8 months	7.6 (5.9-9.9)	N/A	7.6 (6.1-9.7)	N/A
13 months	N/A	12.7 (11.7-14.3)	12.7 (11.9-14.0)	13.0 (12.2-13.6)
Disease severity				
Severe	190 (15%)	149 (15%)	47 (13%)	39 (50%)
Mild	1102 (85%)	846 (85%)	320 (87%)	39 (50%)

infection (16–18), long-term neutralization is thus driven by IgG antibodies to the spike protein (16).

SARS-CoV-2 is constantly mutating yet most changes have little or no impact on its virulence (19). However, some changes are causing concerns regarding disease severity, viral transmissibility, and potential escape from natural and vaccine-induced immunity (20). The World Health Organization (WHO) in collaboration with an international network of experts has characterized the variants of concern (VOC) (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/). Reduced NAb levels as compared to the WT virus have been shown against VOCs, especially against the Beta variant, both after vaccination (13, 21–23) and 9 (13) and 12 months (12) after infection. A similar reduction in NAb titers has also been reported against the Delta variant from convalescent sera collected 3–12 months post symptoms or after vaccination (24, 25).

Previous infection with SARS-CoV-2 has shown to induce effective immunity and protection against reinfections in most individuals (26, 27). In animal studies, a protective antibody titer against SARS-CoV-2 infection has been suggested to be low (28, 29). Higher IgG antibody levels against SARS-CoV-2 among health care workers within three months after vaccination were found to be associated with lower infectivity (30). However, a protective threshold for humans is still under debate and subject to the standardization of serological methods. The accumulating research data on the persistence of antibodies after natural infection, and NAbs in particular, will provide important insight into estimating for how long antibodies induced by Coronavirus disease 2019 (COVID-19) vaccination can be expected to persist and provide protection against emerging SARS-CoV-2 variants. In this study, we investigated the antibody persistence up to 14 months after natural SARS-CoV-2 infection and assessed the potential cross-protection by comparing the NAb levels of WT virus (B lineage) to three VOC strains Alpha (B.1.1.7), Beta (B.1.351), and Delta (B.1.617.2).

Results

Persistence and kinetics of SARS-CoV-2 antibodies

We first assessed the persistence of NAb and serum IgG antibodies specific to SARS-CoV-2 Spike full length (SFL)-IgG, RBD-IgG, and N-IgG at 8 months following SARS-CoV-2 infection. We found that 89% (1148/1292) of the subjects had NAb against the WT virus, 96% (1240/1292) had antibodies to SFL and RBD (S-IgG) and 66% (846/1292) had N-IgG. We further assessed the persistence of NAb and IgG antibodies a year after SARS-CoV-2 infection by randomly selecting 367 of 652 subjects who had not received a SARS-CoV-2 vaccination of the 995 subjects who participated at both time points (Fig. 1). Participant demographics and clinical characteristics for the selected cohort were similar to the overall cohort (Table 1). NAb, S-IgG, and N-IgG antibodies were detected in 91%, 98%, and 67% of subjects in the selected cohort at 8 months after infection, respectively (Table 2). One year after infection the proportion of positive samples was still high for NAb and S-IgG (89% (326/367) and 97% (356/367)), respectively, but had decreased to 36% (132/367) for N-IgG. The mean IgG concentrations decreased significantly ($p < 0.001$) for SFL-IgG, RBD-IgG, and N-IgG from 8 months (3.2, 2.3, 1.2 binding antibody unit concentrations (BAU)/ml) to 13 months (2.3, 1.7, 0.44 BAU/ml, respectively) after infection. The decrease in mean IgG concentration was more notable (-63%) for N-IgG compared to SFL-IgG (-28%) or RBD-IgG (-26%) (Fig. 2).

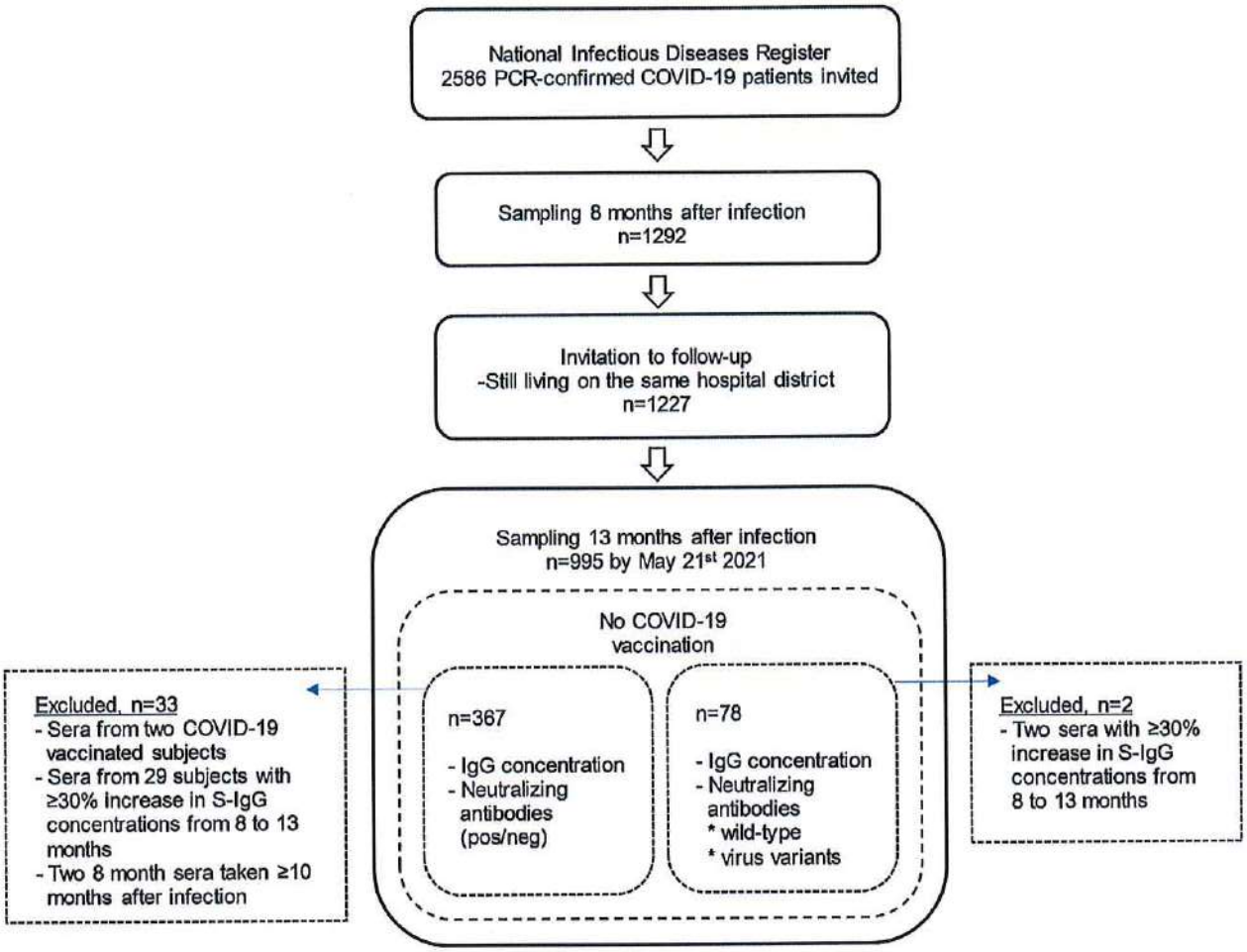

Figure 1. The study flow chart showing the selection of serum samples of the study participants for the determination of antibody concentration and neutralizing antibodies 8 and 13 months after infection.

Effect of disease severity, age, and gender on SARS-CoV-2 antibodies

We observed higher mean N-IgG, SFL-IgG, and IgG-RBD concentrations in subjects who had recovered from severe disease than in those with mild disease 8 months after infection ($p < 0.001$; Fig. 3). The difference was 2.0- to 7.4-fold, depending on the age group, and persisted for at least 13 months after infection (Fig. 3, Table 3). The proportion of seropositive subjects remained high for S-IgG and NAb (100%) and relatively high for N-IgG (67%)

Table 2. Number and proportion of positive samples for spike protein IgG (S-IgG) and neutralizing antibodies (NAb) by disease severity, age and gender of the participants 8 and 13 months after infection, n=367

			S-IgG positive n/n (%)				NAb positive (wt) n/n (%)			
Disease severity	Age (years)	Gender	8 months		13 months		8 months		13 months	
Severe	≥60	M	16/16	(100)	16/16	(100)	16/16	(100)	16/16	(100)
		F	18/18	(100)	18/18	(100)	17/18	(94)	18/18	(100)
	<60	M	6/6	(100)	6/6	(100)	6/6	(100)	6/6	(100)
		F	7/7	(100)	7/7	(100)	7/7	(100)	7/7	(100)
Mild	≥60	M	120/122	(98)	117/122	(96)	105/122	(86)	99/118	(84)
		F	166/171	(97)	165/171	(97)	159/171	(93)	151/171	(88)
	<60	M	15/15	(100)	15/15	(100)	15/15	(100)	14/15	(93)
		F	12/12	(100)	12/12	(100)	10/12	(83)	12/12	(100)

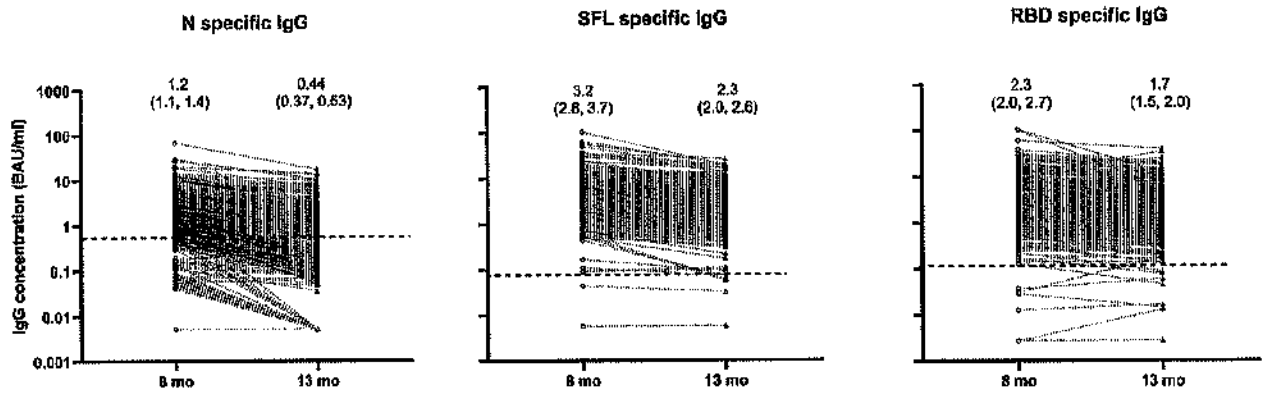

Figure 2. Nucleoprotein (N), and spike protein (SFL, RBD) specific IgG concentrations (BAU/ml) with geometric mean concentrations (95% CI) at 8 and 13 months after infection, n = 367 subjects. FMIA specific cut-off for seropositivity is indicated by a dashed red line. Each sample was tested as technical duplicates in each experiment and the experimental precision was confirmed by two control samples in each independent experiment.

a year after severe infection, compared to 97%, 87%, and 32%, respectively, of those with a milder infection. A higher proportion (33%) of subjects in the elderly age group (≥60 years of age) had been hospitalized compared to the younger age groups (13% of 40 to 59 years and 6% of those 17 to 39 years of age). Elderly subjects (≥60 years of age) with mild infection had similar levels of S-IgG antibodies (Table 3) and an equally high proportion of them had NAb compared to younger subjects with mild infection. N-IgG concentrations were, however, higher among ≥60-year old subjects than in subjects <60 years of age with a mild disease at 8 and 13 months after infection ($p < 0.01$). We could not demonstrate any difference in N-, SFL-, or RBD-IgG concentrations between males and females at 8 or 13 months after infection.

Comparison of NAb titers between a WT virus and three VOCs

A smaller age- and gender-matched subset of participants (n = 78) of 13-month samples was randomly selected for NAb titration due to the laborious live-virus microneutralization test (MNT). The samples were re-analyzed against a WT virus isolated in Finland during 2020 and three VOCs (Alpha, Beta, and Delta) isolated in Finland during 2021. The samples to be included in the NAb titration were selected based on a seropositive result (NAb titer ≥6) in the screening test.

Within the whole cohort (n = 78), NAb titers were significantly lower for all VOCs ($p < 0.0001$, Kruskal-Wallis test) compared to WT virus. This decrease in geometric mean titers (GMT) was more notable for the Beta (-77%) and Delta (-69%) variants than for the Alpha variant (-42%) (Table 4). NAb titers for all VOCs correlated well with WT virus titers, yet a more pronounced correlation was seen for the Alpha and Delta variants and lower for the Beta variant (Supporting information Fig. 1).

For both WT virus and the Alpha variant, the proportion of seropositive individuals with severe disease remained high 13 months after infection (Fig. 4, Supporting information Table 1). Lower titers against the Alpha variant compared to the WT virus were seen in mild disease groups with an increasing proportion of low positive (borderline) or negative subjects. The greatest

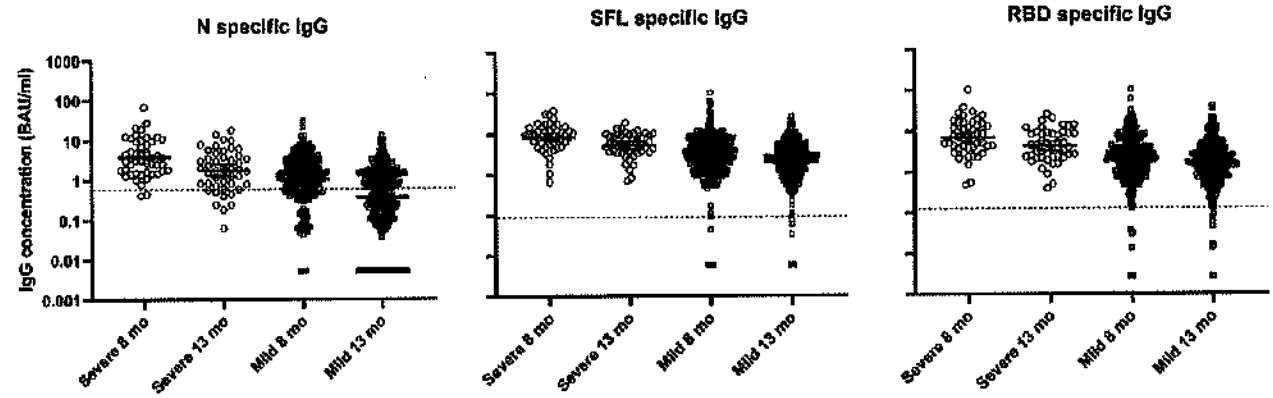

Figure 3. Distribution and the geometric mean of IgG concentrations (BAU/ml and 95% CIs) for nucleoprotein (N specific IgG), and spike protein (SFL and RBD specific IgG) in subjects 8 and 13 months after severe (n = 47 subjects) or mild (n = 320 subjects) infection. FMIA specific cut-off for seropositivity is indicated by a dashed red line. Each sample was tested as technical duplicates in each experiment and the experimental precision was confirmed by two control samples in each independent experiment.

Table 3. Geometric mean IgG concentrations, GMC [95% CI], expressed as BAU/ml for nucleoprotein (N), spike proteins (SFL and RBD) at 8 and 13 months after COVID-19 infection per age group and disease severity. Significantly higher (Kruskal-Wallis test, $p<0.05$) IgG concentrations in subjects with severe as compared to mild disease within age groups are shown in bold

Disease	Age (years)n	N-IgGGMC [95% CI]		RBD-IgGGMC [95% CI]		SFL-IgGGMC [95% CI]	
		8 months	13 months	8 months	13 months	8 months	13 months
Mild	17-39	0.71	0.23	1.7	1.5	2.5	2.0
	n=101	[0.40–1.0]	[0.0061–0.46]	[0.87–2.5]	[0.85–2.1]	[1.1–4.0]	[1.4–2.6]
	40-59	1.2	0.41	2.0	1.5	2.8	1.9
	n=192	[0.74–1.6]	[0.18–0.64]	[0.68–3.4]	[0.83–2.1]	[1.4–4.1]	[1.4–2.5]
	≥60	2.1	0.81	3.0	1.9	4.0	2.6
	n=27	[0.32–3.9]	[-0.29 –1.9]	[1.1–4.8]	[-0.70–4.5]	[1.9–6.1]	[1.1–4.2]
Severe	17-39	2.9	**1.7**	8.4	4.6	6.9	5.1
	n=6	[-1.9–7.7]	[-0.33–3.7]	[-33–50]	[0.19–9.0]	[0.35–14]	[0.82–9.4]
	40-59	**3.9**	**1.8**	**6.2**	**4.0**	**7.6**	**4.7**
	n=28	[-0.96–8.7]	[0.29–3.2]	[3.5–8.8]	[1.8–6.2]	[5.2–10]	[3.2–6.3]
	≥60	4.1	1.8	8.4	4.5	11.4	6.4
	n=13	[-1.4 –9.5]	[-0.91–4.5]	[1.4–15]	[0.89–8.1]	[5.2–18]	[4.1–8.7]

decrease of NAb titers was seen between the WT virus and the Beta variant with markedly lower GMTs and seropositivity with several borderline titers also in groups of severe disease. NAb titers and seropositivity for the Delta variant were also markedly lower compared to WT virus. The Delta GMT values were placed between the GMTs of the Alpha and Beta variants, yet the seropositivity of severe disease groups was relatively well preserved (≥80%) compared to that of the Beta variant (65%).

Table 4. Geometric mean IgG concentrations, GMC [95% CI] expressed as BAU/ml for nucleoprotein (N) and spike proteins (SFL and RBD) and geometric mean titers, GMT [95% CI] of neutralizing antibodies (NAb) against wild-type (wt) virus and three variants of concern Alpha (B.1.1.7), Beta (B.1.351) and Delta (B.1.617.2) 13 months after infection (n=78)

				IgG concentration (BAU/ml)			MNT titer			
Disease severity	Age	Gender	n	N-IgG	S-IgG (RBD)	S-IgG (SFL)	NAb wt	NAb Alpha	NAb Beta	NAb Delta
Severe	<60y	M+F	22	1.5 [0.88-2.7]	3.9 [2.5-6.1]	4.7 [3.0-7.2]	27 [17-41]	21 [14-34]	8.1 [5.0-13]	10 [7.1-15]
		M	12	2.0 [0.99-4.0]	4.7 [2.4-9.0]	5.5 [2.9-10.4]	29 [16-55]	26 [14-49]	9.2 [4.6-19]	14 [8.1-23]
		F	10	1.1 [0.45-2.9]	3.2 [1.8-5.7]	3.8 [2.1-6.8]	24 [12-47]	17 [8.5-32]	6.8 [3.6-13]	7.7 [4.5-13]
	≥60y	M+F	17	1.6 [0.98-2.5]	5.1 [3.0-8.7]	7.6 [4.8-12]	52 [39-71]	30 [20-44]	8.0 [5.1-13]	15 [10-22]
		M	8	0.89 [0.60-1.3]	4.2 [2.2-8.0]	5.8 [3.6-9.2]	39 [27-57]	28 [18-42]	9.2 [4.8-18]	13 [8.5-21]
		F	9	2.6 [1.3-5.0]	6.1 [2.6-14]	9.7 [4.6-21]	68 [45-100]	32 [16-61]	7.0 [3.6-14]	16 [8.6-31]
Mild	<60y	M+F	22	0.41 [0.22-0.75]	1.6 [1.3-2.1]	2.3 [1.9-2.9]	15 [12-20]	8.0 [5.4-12]	3.6 [2.7-4.8]	4.0 [2.8-5.7]
		M	12	0.36 [0.14-0.93]	1.3 [0.89-1.8]	1.8 [1.4-2.4]	12 [9.5-16]	5.1 [3.1-8.4]	2.9 [2.1-4.1]	2.9 [2.0-4.0]
		F	10	0.47 [0.22-1.0]	2.2 [1.6-3.0]	3.1 [2.3-4.0]	20 [14-30]	13 [8.3-22]	4.6 [2.9-7.3]	6.0 [3.4-11]
	≥60y	M+F	17	0.50 [0.26-1.1]	1.8 [1.0-3.1]	2.1 [1.3-3.4]	19 [11-31]	8.5 [4.8-15]	4.2 [2.8-6.5]	5.6 [3.5-8.8]
		M	8	0.94 [0.42-2.1]	1.5 [0.72-3.2]	1.5 [0.82-2.8]	12 [6.1-23]	4.6 [2.2-9.7]	2.9 [1.8-4.6]	4.1 [2.2-7.6]
		F	9	0.28 [0.081-0.98]	2.1 [0.91-4.7]	2.9 [1.5-5.7]	28 [14-55]	15 [7.1-30]	6.0 [3.2-11]	7.4 [3.8-14]

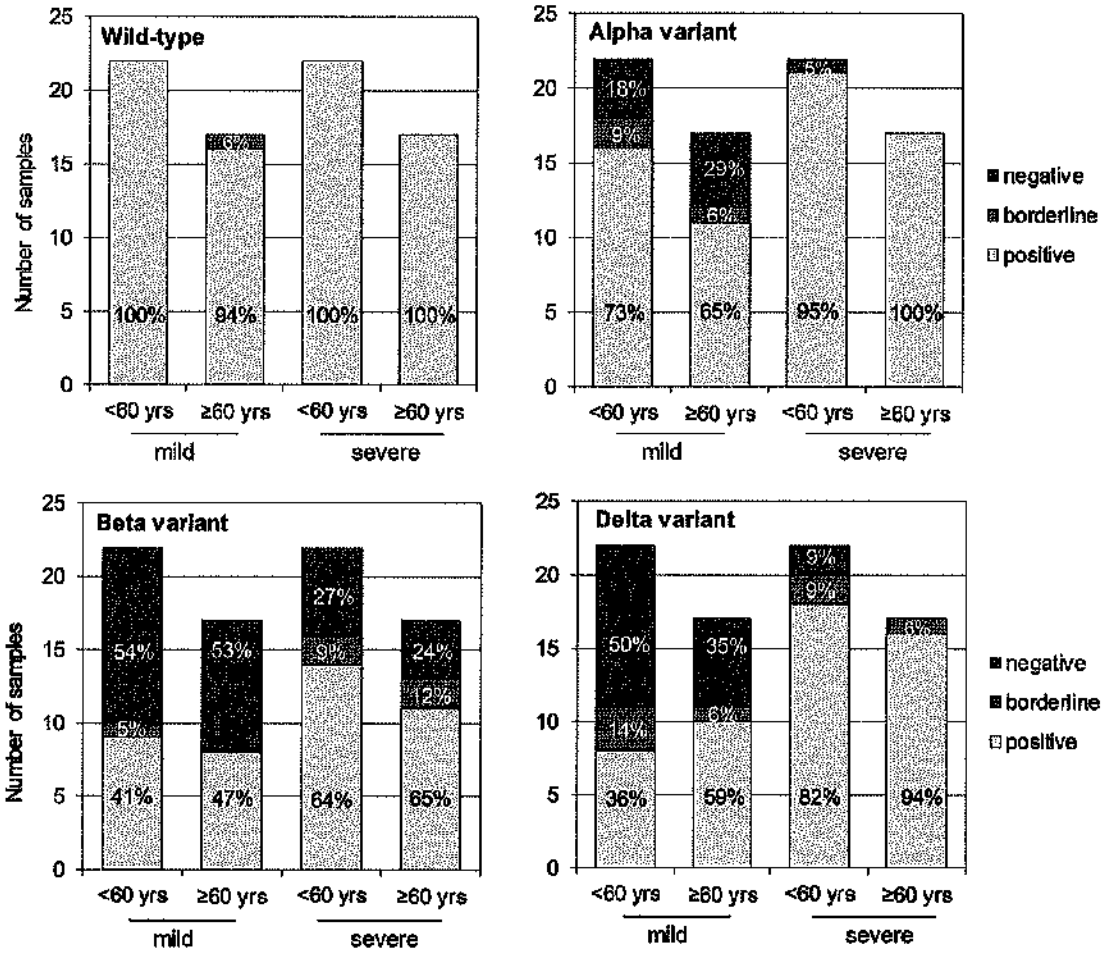

Figure 4. The proportion of subjects positive, low positive (borderline), and negative for neutralizing antibodies 13 months after infection against four SARS-CoV-2 virus strains (n = 78 subjects): The WT virus (B), the Alpha variant (B.1.1.7), the Beta variant (B.1.351), and the Delta variant (B.1.617.2). Each sample was tested as technical duplicates in each experiment and the experimental precision was confirmed by two control samples in each independent experiment.

For all viruses, the subjects who recovered from the severe disease had overall 2.1 to 3.0-fold higher NAb titers compared to those with mild disease ($p < 0.01$). The same finding was seen with all IgG concentrations. The difference in IgG concentrations between severe and mild disease was prominent in both sexes in the large study cohort ($n = 367$). However, in the small cohort ($n = 78$) only males with a mild disease had markedly lower NAb titers and S-IgG concentrations compared to those recovered from severe disease ($p < 0.05$; Supporting information Table 2). The difference was not statistically significant for females although the trend was similar.

NAb titers against WT virus were higher in the elderly group (≥60 years) compared to <60 years old ($p = 0.045$) whereas NAb titers for VOCs did not differ significantly between age groups (Supporting information Table 3). We detected a strong and statistically significant correlation ($p < 0.0001$) between NAb titers and S-IgG antibody concentrations indicating an overall parallel trend between severe and mild disease antibody levels (Fig. 5).

Discussion

Studies of individuals who have recovered from SARS-CoV-2 infection are crucial in determining for how long antibodies persist after infection and whether these antibodies protect against re-infection. We showed that S-IgG antibodies and, most importantly, NAbs persist in most subjects for at least a year following SARS-CoV-2 infection. The concentration of N-IgG, on the contrary, declined among a large proportion of subjects. In accordance with previous observations (6, 8, 31), subjects with severe infection had higher N-IgG, S-IgG concentrations, and NAb titers than subjects with mild infection and are expected to remain seropositive for a longer time.

Previous studies show that most patients recovering from COVID-19 have detectable antibody responses peaking at approximately one month after infection (7, 8, 32). Antibody levels to N and S protein antigens decline during the first few months with differences in isotype and antigen specificity of the antibody (7). The decay rate has been shown to slow down thereafter (12).

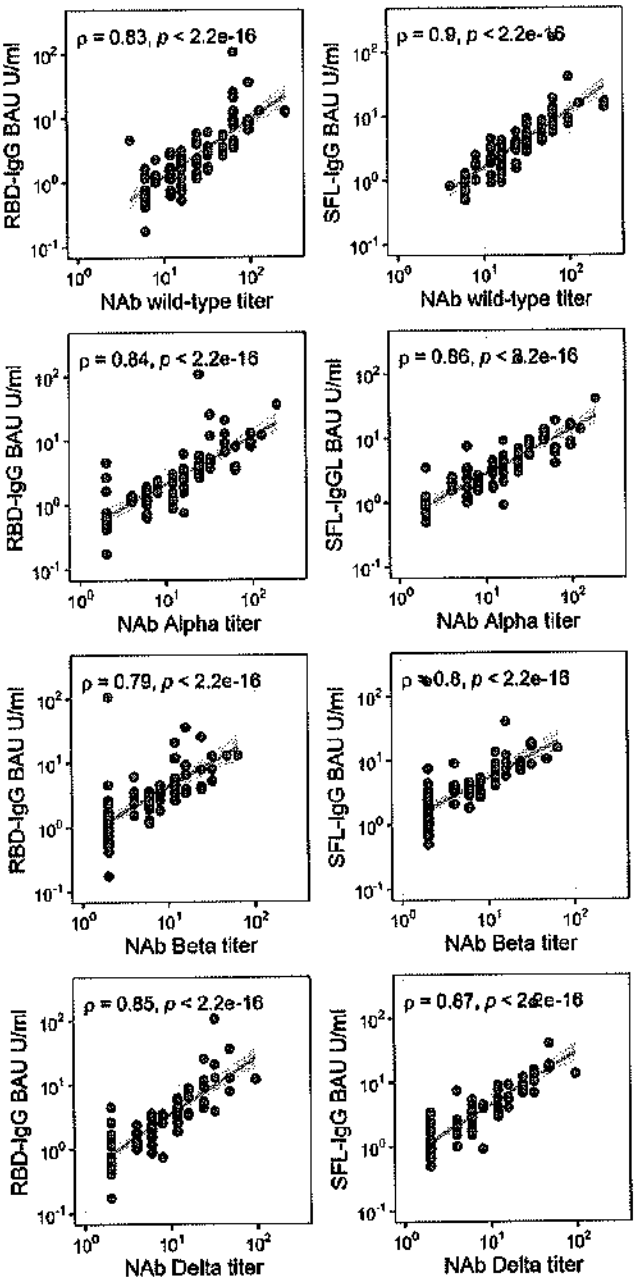

Figure 5. Spearman correlation (ρ) and significance (p) between S-IgG antibody concentrations and neutralizing antibody (NAb) titers against the WT virus (B) and the variants of concern: Alpha (B.1.1.7), Beta (B.1.351), and Delta (B.1.617.2). One point may represent multiple samples (n = 78 subjects). Each sample was tested as technical duplicates in each experiment and the experimental precision was confirmed by two control samples in each independent experiment.

The relatively rapid early decline in S-IgG antibodies followed by slower decay indicates a transition of serum antibodies from being produced by short-lived plasmablasts to a more persistent population of long-lived plasma cells generated later in the immune response (33). Consistently, NAbs and T cell immunity have been reported to persist at least 6 to 12 months after infection (6-8, 11–13, 34). Our data are consistent with previous data suggesting that, even though NAb titers decline with time, NAbs persist in most subjects, at least up to 13 months.

We observed that a markedly lower proportion of subjects had N-IgG than S-IgG antibodies at 8 months after infection. Thereafter the concentration of N-IgG antibodies declined to a level that was not distinguishable from unspecific, cross-reactive antibodies among a large proportion of subjects 13 months after infection. SARS-CoV-2 N is produced abundantly during infection and since it is not a component in present vaccines or vaccine candidates it could potentially serve as a measure of past infection. However, our results clearly show that the sensitivity of our N-IgG-based antibody assay is inversely proportional to the time after infection. In agreement with our findings, the more rapid decay of N-IgG after SARS-CoV and SARS-CoV-2 infection has also been reported in other studies (32, 35, 36). The loss of sensitivity of SARS-CoV-2 N based antibody assays over time likely results not only from the decay of the antibodies, but from the difficulty of differentiating very low concentrations of SARS-CoV-2-specific antibodies from cross-reactive N antibodies induced by past infections with common cold human coronaviruses that share highly conserved regions (37).

Even though NAbs persist relatively long in most subjects, neutralization efficiency against the Alpha (B.1.1.7), Beta (B.1.351), and Delta (B.1.617.2) variants was decreased compared to the WT virus. This was emphasized in subjects who had recovered from mild disease representing the majority of COVID-19 cases (1). Indeed, mild symptomatic or asymptomatic individuals may develop no or only low levels of NAbs that may wane relatively quickly after infection (38).

In line with earlier observations 9 (13) and 12 months after infection (12), we found that NAb levels against the Alpha variant were only slightly reduced, while NAb levels against the Beta variant were considerably declined compared to the WT virus. The Beta variants have been shown to evade antibody responses induced upon infection as well as vaccination (21-23, 39, 40). Although the NAb levels were declined against the Beta variant, we observed that over 60% of hospitalized subjects were seropositive a year after infection, indicating long-lived cross-neutralization capacity induced by severe disease.

We detected substantially declined NAb titers against the Delta variant in subjects with mild disease, similar to what has been previously reported after vaccination or up to 12 months after SARS-CoV-2 infection (24, 25, 41–43). However, we observed that over 80% of the subjects who had recovered from a severe disease were seropositive against the Delta variant. This is in line with one study reporting only modestly reduced (88%) NAb levels against the Delta variant 2–4 weeks after second vaccine dose (44). Our results support the previous findings that the emerging variant Delta partially but significantly escapes NAbs (24, 25).

One previous study reported lower seropositivity rates one year after mild SARS-CoV-2-infection compared to our results; 58% were positive for S1-IgG and 85% for S-IgG measured with enzyme immunoassay and 58% had NAb (11). Direct comparison of the IgG concentrations and NAb titers between studies may not be possible since the age groups, viruses, as well as the serological

tests, differed. Neutralizing antibody tests have not been standardized and among other things, the starting dilutions of serum samples may vary between assays. The microneutralization assay used in this study utilized live virus and the starting dilution of 1:4 further enhances the sensitivity of the assay in detecting low levels of NAbs.

In our study population, we could not see a gender effect in hospitalized individuals, as previously reported (6, 31). However, hospitalized subjects ≥60 years tended to have slightly higher IgG and NAb levels compared to hospitalized subjects <60 years suggesting more severe infection in the elderly age group. Although there was no overall difference between the genders, especially males with mild disease had markedly lower NAb titers for all viruses compared to individuals who recovered from severe disease.

There is a major research effort to produce effective SARS-CoV-2 vaccines. The long-term persistence of immunity after vaccination is, however, largely unknown. Evidence from convalescent sera from individuals who have recovered from infection may help determine for how long immunity persists, and whether antibodies might protect against re-infection. Previous data shows that, when measured as IgG antibodies against *S* protein or RBD and NAb, immune response after two doses of SARS-CoV-2 vaccine is similar to that observed in convalescent sera from COVID-19 patients (45–48). Evidence of persistence of immunity after infection will help in predicting the persistence of immunity after SARS-CoV-2 vaccination.

We recognize certain limitations in our study. Due to high SARS-CoV-2 vaccine coverage in the older age groups (≥60 years of age) at the time of our study, only 11% of the participants were ≥60 years of age, the age group with the highest disease incidence and morbidity. Our results may not necessarily apply to all age groups. The number of subjects selected for the NAb titer comparison was limited but the study subjects were matched by disease severity, age, and gender, and randomly selected from the participants.

Previous studies have indicated that the presence of antibodies to SARS-CoV-2 was associated with a significantly reduced risk of SARS-CoV-2 reinfection among healthcare workers for up to 7 months after infection (27, 49). We observed that S-IgG antibodies and NAbs persist at least a year after infection in most individuals. This strongly suggests that protection against re-infection is long-lived, although antibody-mediated immunity may not persist equally well among elderly subjects. A previous study found that patients >60 years had fewer memory B cells secreting total IgG and RBD-specific IgG than patients <60 years old 9 months after infection (9). We observed that IgG concentrations declined from 8 to 13 months more substantially in subjects ≥60 years compared to younger age groups. A similar more rapid decline in NAb concentrations was observed among the elderly compared to younger subjects who were followed up to 6 months following vaccination (50). The results of our study support previous findings indicating that protection against infection mediated by NAbs may be impaired against the VOCs, especially after a mild disease. While in the absence of NAbs reinfection is possible, cellular immunity is not similarly affected by mutations in the RBD site (22) and is likely to provide long-term protection against severe disease.

Materials and methods

Study design and participants

In October 2020, 2586 subjects ≥18 years of age, native language Finnish or Swedish, living within five selected hospital districts in Finland and with a PCR-confirmed COVID-19 diagnosis between February 29 and April 30, 2020 were identified in the National Infectious Disease Register and invited to participate in the follow-up study. Subjects within institutional care were excluded. Informed consent was obtained from all study subjects before sample collection. A total of 1292 (50%) subjects (median age 50.0, range 17.3-94.3) with PCR-confirmed COVID-19 participated and donated a blood sample for determination of SARS-CoV-2 specific serum antibodies 5.9 to 9.9 months (median 7.6 months) after infection. All those previously enrolled and still living in the same hospital district (n = 1227) were invited to a follow-up visit and blood sampling a year after the COVID-19 diagnosis in March-April 2021. By May 21, 2021, altogether 995 participants (median age 52.5, range 17.6-95.6 years) had participated at 12.7 months (median, range 11.7 to 14.3 months) after the diagnosis of PCR-confirmed COVID-19. Demographics, clinical characteristics, and SARS-CoV-2 vaccination history of the participants were collected from the National Infectious Disease Register, the Care Register for Health Care, the Register of Primary Health Care Visits, and the National Vaccination Registry and are summarized in Table 1. The disease severity was defined as severe or mild. Severe infection was defined as an individual with laboratory-confirmed COVID-19 and who required hospital treatment. Mild infection was defined as an individual with laboratory-confirmed COVID-19 without hospital treatment. Since late December 2020 SARS-CoV-2 vaccinations have been offered according to the national recommendations in Finland.

Sample processing and selection of samples

Sera were separated by centrifugation, aliquoted, and stored at -20°C or below. For assessment of NAbs, sera were heat-inactivated (56°C for 30 min) and then stored at -20°C or below.

For assessment of persistence of serum antibodies 8 months following PCR-confirmed COVID-19 diagnosis, all samples taken ≤10 months after diagnosis (n = 1292) were selected for assessment of SARS-CoV-2 IgG antibody concentration and NAbs (positive/borderline/negative). For assessment of antibody persistence 13 months after infection, 400 of 995 sera were randomly selected for determination of SARS-CoV-2 IgG antibody concentration and NAbs. Selection criteria were: 8-month sample available,

PCR-confirmed COVID-19 diagnosis, no documentation of SARS-CoV-2 vaccination in the Register of Primary Health Care Visits by June 10th 2021. Further, samples of subjects with ≥30% increase in IgG antibody concentration to both SARS-CoV-2 S gp antigens (full-length spike protein (SFL) and RBD) between 8- and 13-month blood sampling (n = 29) were excluded from the analysis. An additional four samples were excluded due to the late discovery of these samples not meeting selection criteria. Of the four samples, two were excluded due to vaccination and two due to samples taken >10 months after infection. Consequently, 367 sera were selected.

For comparison of NAb titers against a WT virus and VOCs (Alpha, Beta, and Delta), 80/536 13-month sera screened to NAb (titer ≥6 against WT virus) were randomly selected as mentioned above. Later observed ≥30% increase in IgG antibody concentration between 8- and 13-month samples excluded two of 80 samples, leaving total sample size to 78. SARS-CoV-2 IgG antibody concentration was measured from this cohort to ensure its comparability to the other 367 sera selected.

SARS-CoV-2 MNT

A cytopathic effect-based MNT was performed as previously described (51, 52). Briefly, heat-inactivated serum samples were 2-fold serially diluted starting from 1:4 in Eagle's MEM supplemented with penicillin, streptomycin, and 2% of heat-inactivated fetal bovine serum. At the biosafety level 3 laboratory, pre-titrated virus was added to obtain 100× tissue culture infectious dose 50% per well following incubation for 1 h at +37°C, 5% CO_2. African green monkey kidney epithelial (VeroE6) cells were added and the 96-well tissue culture plates were incubated at +37°C, 5% CO_2 for 4 days. Wells were fixed with 30% formaldehyde and stained with crystal violet. Results were expressed as MNT titers corresponding to the reciprocal of the serum dilution that inhibited 50% of SARS-CoV-2 infection observed by the cytopathic effect of inoculated cells. MNT titer ≥6 was considered positive, borderline when 4, and negative when <4. Borderline values were further confirmed with biological repeats. For titer comparison, a titer of 192 was measured for the WHO International Standard (NIBSC 20/136 (53)) using the WT virus Fin1-20.

SARS-CoV-2 viruses selected for MNT

All samples were screened with WT virus Fin1-20 (B lineage): hCoV-19/Finland/1/2020 (GISAID accession ID EPI_ISL_407079; GenBank accession ID MZ934691) for NAb positivity. Fin1-20 was the first SARS-CoV-2 strain detected in Finland in January 2020. Virus isolation and propagation were performed in Vero E6 cells (51). A smaller subset of samples was analyzed also with VOCs isolated in Finland during January 2021: Fin34-21, Fin32-21, and May 2021: Fin37-21, which stand for the Alpha, Beta, and Delta variant, respectively. Alpha variant (B.1.1.7) Fin34-21 indicates the isolate hCoV-19/Finland/THL-202102301/2021 (EPI_ISL_2590786; MZ944886). Spike region of the isolate hCoV-19/Finland/THL-202101018/2021 (Fin32-21) showed typical Beta variant (B.1.351) amino acid changes (EPI_ISL_3471851; MZ944846). The Delta variant (B.1.617.2) Fin37-21 indicates hCoV-19/Finland/THL-202117309/2021 (EPI_ISL_2557176; MZ945494). All variant viruses were isolated and propagated (passages 1–2) in VeroE6-TMPRSS2-H10 cells (54) and further propagated in Vero E6 cells (passage 3) for MNT.

SARS-CoV-2 fluorescent multiplex immunoassay

The SARS-CoV-2 fluorescent multiplex immunoassay (FMIA) has been previously described in detail by Ekström et al. (52) and Solastie et al. (55). Briefly, diluted sera, reference, and controls were mixed with microspheres conjugated with SARS-CoV-2 N and SFL and RBD of the spike protein. IgG antibodies were detected by R-Phycoerythrin-conjugated secondary antibody and median fluorescence intensity was measured with MAGPIX system (Luminex) and BAU (U/ml) were interpolated from 5-parameter logistic curves with xPONENT (v. 4.2, Luminex) created by 7-point serial fourfold diluted reference sera calibrated against WHO International Standard (NIBSC code 20/136; (53)). When the median fluorescence intensity of a sample was below the linear range of the reference, the sample was assigned an antibody concentration half of the limit of detection (0.0094, 0.012, and 0.0057 BAU/ml for N-, SFL-, and RBD-IgG). A sample was considered positive for SARS-CoV-2 S-IgG when SFL and RBD specific antibody concentrations were ≥0.089 and ≥0.13 BAU/ml, respectively. A sample was considered positive for N-IgG when N-IgG concentration was ≥0.58 BAU/ml. The cut-offs for seropositivity were determined during clinical validation of the FMIA and yielded both sensitivity and specificity of 100% for SFL- and RBD-IgG and 98.6% and 100% for N-IgG for samples taken 13 to 150 days post-onset of symptoms, respectively (52, 55).

Statistical methods

We calculated the geometric mean concentrations (GMC) and GMTs with 95% confidence intervals (CI) for IgG and NAb levels, respectively. We assessed the statistical differences in antibody levels between groups using the Kruskal-Wallis test with Bonferroni correction. Differences in mean IgG concentrations between 8 and 13 months after infection were compared using Student's paired *t*-test and log-transformed data. The statistical significance level of difference was set to $p<0.05$. We used Spearman correlation in the correlation analyses. MNT titers <4 were assigned a titer value of 2. Samples with IgG concentrations below the limit of detection were assigned an antibody concentration equal to half of the limit of detection. Statistical analyses were performed using SPSS v27 and R (v4.0.4) with Rstudio (v1.4.1106).

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Ethics approval and patient consent statement: The study protocol was approved by the ethical committee of the Hospital District of Helsinki and Uusimaa and registered under the study protocol HUS/1137/2020. Informed consent was obtained from all study subjects before sample collection.

Author contributions: M.M., N.E., and A.H. designed the experiments. M.M., A.A.P., and H.N. contributed to the study design. C.V. and A.S. developed and performed the FMIA tests. A.H. developed and performed the microneutralization tests. P.Ö. coordinated the virus isolations. A.H., N.E., and A.S. analyzed the data. E.I. and N.E coordinated the participant recruitment, sample collection, and sample processing. A.H., N.E., and M.M. wrote the manuscript and all co-authors contributed to the edition of the text.

Conflict of interest: Finnish Institute for Health and Welfare has received research funding for unrelated studies from GlaxoSmithKline Vaccines (N.E., C.V., A.A.P. and M.M. as investigators), Pfizer (A.A.P.), and Sanofi Pasteur (A.A.P.). The other authors report no potential conflicts of interest.

Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request. The complete data are not publicly available due to privacy or ethical restrictions.

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Abbreviations: **BAU**: binding antibody unit concentration · **COVID-19**: Coronavirus Disease 2019 · **FMIA**: fluorescent multiplex immunoassay · **GMC**: geometric mean concentration · **GMT**: geometric mean titer · **MNT**: microneutralization test · **NAb**: neutralizing antibody · **N**: nucleoprotein · **PCR**: polymerase chain reaction · **RBD**: receptor-binding domain · **SARS-CoV-2**: Severe acute respiratory syndrome coronavirus 2 · **S**: spike protein · **SFL**: spike full length · **VOC**: variants of concern

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ORIGINAL ARTICLE

Waning Immune Humoral Response to BNT162b2 Covid-19 Vaccine over 6 Months

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ABSTRACT

BACKGROUND

Despite high vaccine coverage and effectiveness, the incidence of symptomatic infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been increasing in Israel. Whether the increasing incidence of infection is due to waning immunity after the receipt of two doses of the BNT162b2 vaccine is unclear.

METHODS

We conducted a 6-month longitudinal prospective study involving vaccinated health care workers who were tested monthly for the presence of anti-spike IgG and neutralizing antibodies. Linear mixed models were used to assess the dynamics of antibody levels and to determine predictors of antibody levels at 6 months.

RESULTS

The study included 4868 participants, with 3808 being included in the linear mixed-model analyses. The level of IgG antibodies decreased at a consistent rate, whereas the neutralizing antibody level decreased rapidly for the first 3 months with a relatively slow decrease thereafter. Although IgG antibody levels were highly correlated with neutralizing antibody titers (Spearman's rank correlation between 0.68 and 0.75), the regression relationship between the IgG and neutralizing antibody levels depended on the time since receipt of the second vaccine dose. Six months after receipt of the second dose, neutralizing antibody titers were substantially lower among men than among women (ratio of means, 0.64; 95% confidence interval [CI], 0.55 to 0.75), lower among persons 65 years of age or older than among those 18 to less than 45 years of age (ratio of means, 0.58; 95% CI, 0.48 to 0.70), and lower among participants with immunosuppression than among those without immunosuppression (ratio of means, 0.30; 95% CI, 0.20 to 0.46).

CONCLUSIONS

Six months after receipt of the second dose of the BNT162b2 vaccine, humoral response was substantially decreased, especially among men, among persons 65 years of age or older, and among persons with immunosuppression.

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As the rollout of vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1,2] is expanding worldwide, data on the durability of protection are limited. A randomized, controlled trial and real-world studies have shown vaccine efficacy of 94 to 95% with the BNT162b2 vaccine (Pfizer–BioNTech) and vaccine effectiveness in preventing symptomatic coronavirus disease 2019 (Covid-19) 7 days or more after receipt of the second dose of vaccine.[1,3-5] Real-world effectiveness and immunogenicity data describing the antibody kinetics over time after vaccination are beginning to appear, but a complete picture of the duration of immunity is not yet available. We recently reported that breakthrough infection in BNT162b2-vaccinated persons was correlated with neutralizing antibody titers.[6] However, a threshold titer that can predict breakthrough infection has not been defined.

The BNT162b2 vaccine elicits high IgG and neutralizing antibody responses 7 to 14 days after receipt of the second dose. Lower antibody levels have been shown to develop in older persons, men, and persons with an immunosuppressed condition, which suggests that antibody titers in these populations may decrease earlier than in other populations.[7,8] A decrease in anti-spike (S) antibody levels by a factor of two was observed from the peak (at 21 to 40 days) to 84 days after receipt of the second dose of the BNT162b2 vaccine among 197 vaccinated persons.[9] Here, we report the results of a large-scale, real-world, longitudinal study involving health care workers that was conducted to assess the kinetics of immune response among persons with different demographic characteristics and coexisting conditions throughout the 6-month period after receipt of the second dose of the BNT162b2 vaccine.

METHODS

STUDY DESIGN AND POPULATION

We conducted this prospective longitudinal cohort study involving health care workers at Sheba Medical Center, a large tertiary medical center in Israel that includes 1600 beds and 14,739 health care workers, including employees, students, volunteers, and retired personnel. The distribution of the health care workers at Sheba Medical Center is as follows: 18% are physicians, 27% are nurses or nurse aids, 21% are paramedical personnel, and 34% are administrative or logistic employees. The study protocol was approved by the institutional review board at Sheba Medical Center.

All the participants were health care workers who had been invited to participate in the study and provide peripheral-blood samples for serologic assays before receipt of the first vaccine dose and then monthly (every 28 days, within a window of ±14 days) for 6 months after receipt of the second vaccine dose. Written informed consent was obtained from all study participants.

Eligibility criteria included an age of 18 years or older, no SARS-CoV-2 infection before receipt of the first vaccine dose (determined on the basis of either a negative anti–SARS-CoV-2 IgG test or the absence of a positive polymerase-chain-reaction [PCR] assay result for SARS-CoV-2, with no history of suspected clinical SARS-CoV-2 infection), and at least one serologic assay result after receipt of the second dose of vaccine. Data on PCR testing are provided in Supplementary Methods Section S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org. The end of the study for any participant was defined as 175 days after receipt of the second vaccine dose, a positive SARS-CoV-2 PCR or antinucleocapsid (anti-N) antibody result, or loss to follow-up.

All the health care workers at Sheba Medical Center were required to report a daily health status on arrival at the hospital. If any Covid-19–associated symptom or exposure to a SARS-CoV-2–infected person at work, at home, or in the community was reported, a PCR test for SARS-CoV-2 was required.[6,10] In addition, monthly serologic follow-up was conducted during the study period. Participants with a substantial increase in IgG antibody levels or neutralizing antibody titers (≥4 times) between consecutive tests were tested for anti-N antibody to rule out a Covid-19 breakthrough infection and, if positive, were withdrawn from the study.

Participants were notified of their personal test results. Participants whose IgG and neutralizing antibody titers decreased to below the test cutoff level tended not to return for follow-up visits. These and other missing outcomes were accommodated through the linear mixed model that was used in the analysis (described below).

STUDY DESIGN OF SEROLOGIC ASSAYS

Antibodies were tested during the baseline period (defined as days 4 through 17 after receipt of the second vaccine dose) and every 4 weeks thereafter: during days 18 through 42 (period 1), days 43 through 70 (period 2), days 71 through 98 (period 3), days 99 through 126 (period 4), days 127 through 154 (period 5), and days 155 through 175 (period 6). Because we could not perform neutralizing antibody assays in all study participants, we selected a subgroup that included higher proportions of persons with risk factors of interest, such as an age of 65 years or older and coexisting conditions. Criteria for the selection of participants for the neutralizing antibody subgroup are listed in Supplementary Methods Section S2. The peak period was defined as the interval of time with the highest titers after receipt of the second dose.

A correlation between neutralizing antibody titers and infectivity was recently reported[6] and suggested that although the specific threshold titer that can predict breakthrough infection is still undefined, neutralizing antibodies may be used as a correlate of protection. We therefore assessed the probability of having a titer below the cutoff for diagnostic positivity on the neutralizing antibody test (i.e., 16), as well as four titrations above it: 32, 64, 128, and 256. We assessed titers of IgG and neutralizing antibody at two primary time points: the peak period (as defined above) and the end of study (at 175 days).

Data on age and sex were available for all study participants. A computer-based questionnaire about demographic characteristics and coexisting conditions was sent electronically to all study participants. The questionnaire and definitions of the study variables are provided in Tables S2 and S3. Participants who did not respond to the questionnaire were not included in the mixed-model analysis.

SEROLOGIC ASSAYS

Samples from vaccinated participants were tested for antibodies against SARS-CoV-2 receptor-binding domain with the Access SARS-CoV-2 IgG assay (Beckman Coulter).[11,12] Anti-N antibodies were tested with the Platelia SARS-CoV-2 Total Ab Assay (Bio-Rad) according to manufacturer instructions. The SARS-CoV-2 pseudovirus neutralization assay was performed as described previously[7] with the use of a green fluorescent protein reporter–based pseudotyped virus with a vesicular stomatitis virus backbone coated with SARS-CoV-2 S protein. The lower level of diagnostic detection for IgG is 0.62, and the lower level that is considered neutralizing is 16. Additional information about antibody testing is provided in Supplementary Methods Section S3.

STATISTICAL ANALYSIS

We used linear mixed models to examine the IgG and neutralizing antibody kinetics over the 6-month period after receipt of the second vaccine dose and to associate these changes with the demographic characteristics and coexisting conditions of the participants. The dependent variable was either the IgG or neutralizing antibody level, which was log-transformed. Fixed-effect covariates included sex, age group (18 to 44 years, 45 to 64 years, or ≥65 years), and age-by-sex interaction. Time was modeled as a constant up to 30 days after receipt of the second dose and as a linear trend thereafter. For neutralizing antibody levels, the slope of the linear trend was allowed to change at day 70 after receipt of the second dose. Interactions of the initial time slope (from day 30 onward) with age group and sex were also included. In addition to this basic model, body-mass index (BMI; the weight in kilograms divided by the square of the height in meters) and coexisting conditions were added so that we could examine their relationships to antibody kinetics, and interactions of time with each of these covariates were retained in the model only if they were significant at the 5% level after Bonferroni adjustment for multiple comparisons. Individual participant level and time trend were included as random effects. Missing data regarding IgG or neutralizing antibody levels were accommodated within these models under the "missing at random" assumption. Participants with missing values for the covariates were excluded from the analysis. Additional details regarding the mixed-models analysis are provided in Supplementary Methods Sections S4 and S5.

The estimated effects of covariates are presented as ratios of means with 95% confidence intervals on the original scale of the IgG and neutralizing antibodies. A two-sided P value of less than 0.05 was considered to indicate statistical significance. On the basis of each fitted model, the estimated probability of having a titer

below the specified different neutralizing antibody titers at 6 months after receipt of the second dose (with the 95% confidence interval) was calculated for different participant profiles by means of computer simulation.

Scatter plots of log-transformed IgG and neutralizing antibody levels and the distributions of log-transformed IgG and neutralizing antibody levels according to time since the receipt of the second dose were created with the use of GraphPad Prism software, version 5.0 (GraphPad Software). Correlations between IgG and neutralizing antibody levels for each period were assessed by Spearman's rank correlation. Statistical analysis was performed with the use of SAS software, version 9.4 (SAS Institute), and the linear mixed-models analyses were performed with the use of R software, version 3.6.2 (R Foundation for Statistical Computing).

RESULTS

STUDY POPULATION AND SEROLOGIC ASSAYS

The study was conducted from December 19, 2020, to July 9, 2021. Of the 12,603 vaccinated health care workers who were eligible for the study, 4868 were recruited for study participation (Fig. 1). During the study period, 20 participants had a breakthrough SARS-CoV-2 infection (defined as a positive PCR result for SARS-CoV-2), and 5 had a positive anti-N result. A total of 14,736 IgG assays and 4521 neutralizing antibody assays were performed. The numbers of persons with repeated IgG tests and neutralizing antibody assays are shown in Figure 1. IgG levels were evaluated at least once for all study participants during the 6 months of follow-up and at least twice for 2631 participants (54.0%). The neutralizing antibody subgroup included 1269 participants (26.1%) who underwent at least one neutralizing antibody test; 955 of these participants (75.3%) were tested at least twice. Data on age and sex were available for all study participants. Overall, 3808 participants (78.2%) responded to the computer-based questionnaire and were included in the mixed-model analysis.

The demographic characteristics and data on coexisting conditions in the study participants are provided in Table S1, in both the overall population and the neutralizing antibody subgroup. The mean (±SD) age of the participants was 46.9±13.7 years in the overall population and 52.7±14.2 years in the neutralizing antibody subgroup. The distributions of the demographic characteristics and coexisting conditions among the participants according to study period and IgG and neutralizing antibody assays are provided in Tables S4 and S5.

SARS-COV-2 ANTIBODY KINETICS AFTER RECEIPT OF SECOND VACCINE DOSE

Antibody response and kinetics were assessed for 6 months after receipt of the second vaccine dose (Figs. 2A and 2B and S1 and Table S6). The highest titers after the receipt of the second vaccine dose (peak) were observed during days 4 through 30, so this was defined as the peak period. The expected geometric mean titer (GMT) for IgG for the peak period, expressed as a sample-to-cutoff ratio, was 29.3 (95% confidence interval [CI], 28.7 to 29.8). A substantial reduction in the IgG level each month, which culminated in a decrease by a factor of 18.3 after 6 months, was observed. Neutralizing antibody titers also decreased significantly, with a decrease by a factor of 3.9 from the peak to the end of study period 2, but the decrease from the start of period 3 onward was much slower, with an overall decrease by a factor of 1.2 during periods 3 through 6. The GMT of neutralizing antibody, expressed as a 50% neutralization titer, was 557.1 (95% CI, 510.8 to 607.7) in the peak period and decreased to 119.4 (95% CI, 112.0 to 127.3) in period 6.

DIFFERENTIAL DECAY ACCORDING TO AGE AND SEX

IgG and neutralizing antibody kinetics showed differences in immunogenicity according to age group and sex (Fig. 2C through 2F). The rate of IgG decay in all subgroups defined according to age and sex was constant throughout the 6-month period, whereas neutralization was substantially reduced up to period 3, followed by a slower decrease thereafter. Participants 65 years of age or older had lower IgG and neutralizing antibody levels than persons 18 to less than 45 years of age during the peak period and also had a greater decrease, up to approximately 3 months (end of period 2), in the neutralizing antibody titer (Fig. 2C and 2D, and see Supplementary Results Sections S1 and S2).

PREDICTORS OF PEAK AND END-OF-STUDY ANTIBODY TITERS

In the peak and end-of-study periods, significantly lower IgG titers were associated with older

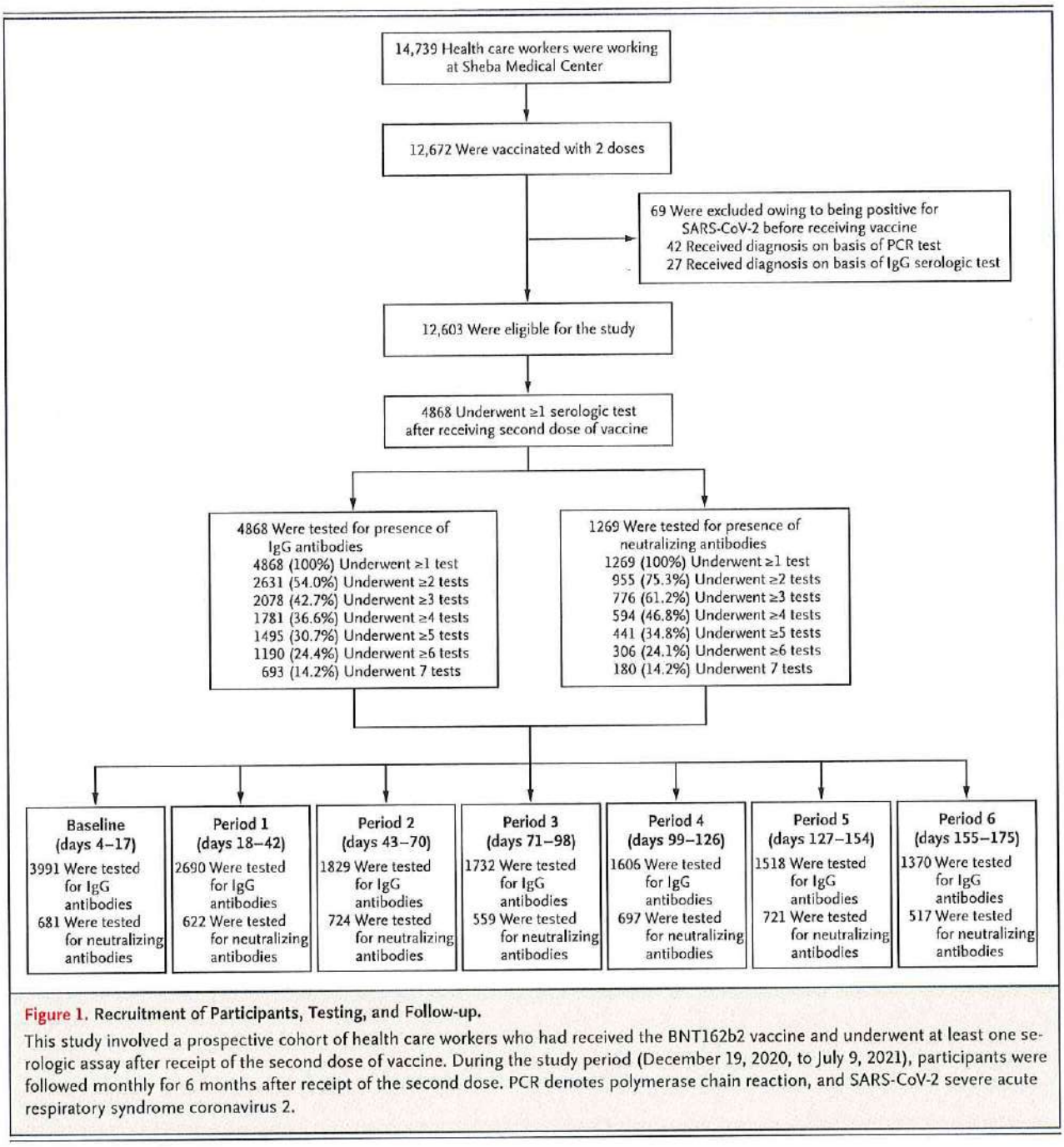

Figure 1. Recruitment of Participants, Testing, and Follow-up.

This study involved a prospective cohort of health care workers who had received the BNT162b2 vaccine and underwent at least one serologic assay after receipt of the second dose of vaccine. During the study period (December 19, 2020, to July 9, 2021), participants were followed monthly for 6 months after receipt of the second dose. PCR denotes polymerase chain reaction, and SARS-CoV-2 severe acute respiratory syndrome coronavirus 2.

age, male sex, the presence of two or more coexisting conditions (i.e., hypertension, diabetes, dyslipidemia, or heart, lung, kidney, or liver disease), the presence of autoimmune disease, and the presence of immunosuppression. Significantly lower neutralizing antibody titers were associated with older age, male sex, and the presence of immunosuppression in both periods, and significantly higher neutralizing antibody titers were associated with a BMI of 30 or higher (obesity) as compared with a BMI of less than 30 in both study periods. Our results show that although the IgG and neutralizing antibody titers were significantly lower in participants with two or more specific coexisting conditions than in those with no specific coexisting condition during the peak period, no significant differences in neutralizing antibody titers were observed at the end of study. In addition, participants with autoimmune disease had a significantly lower

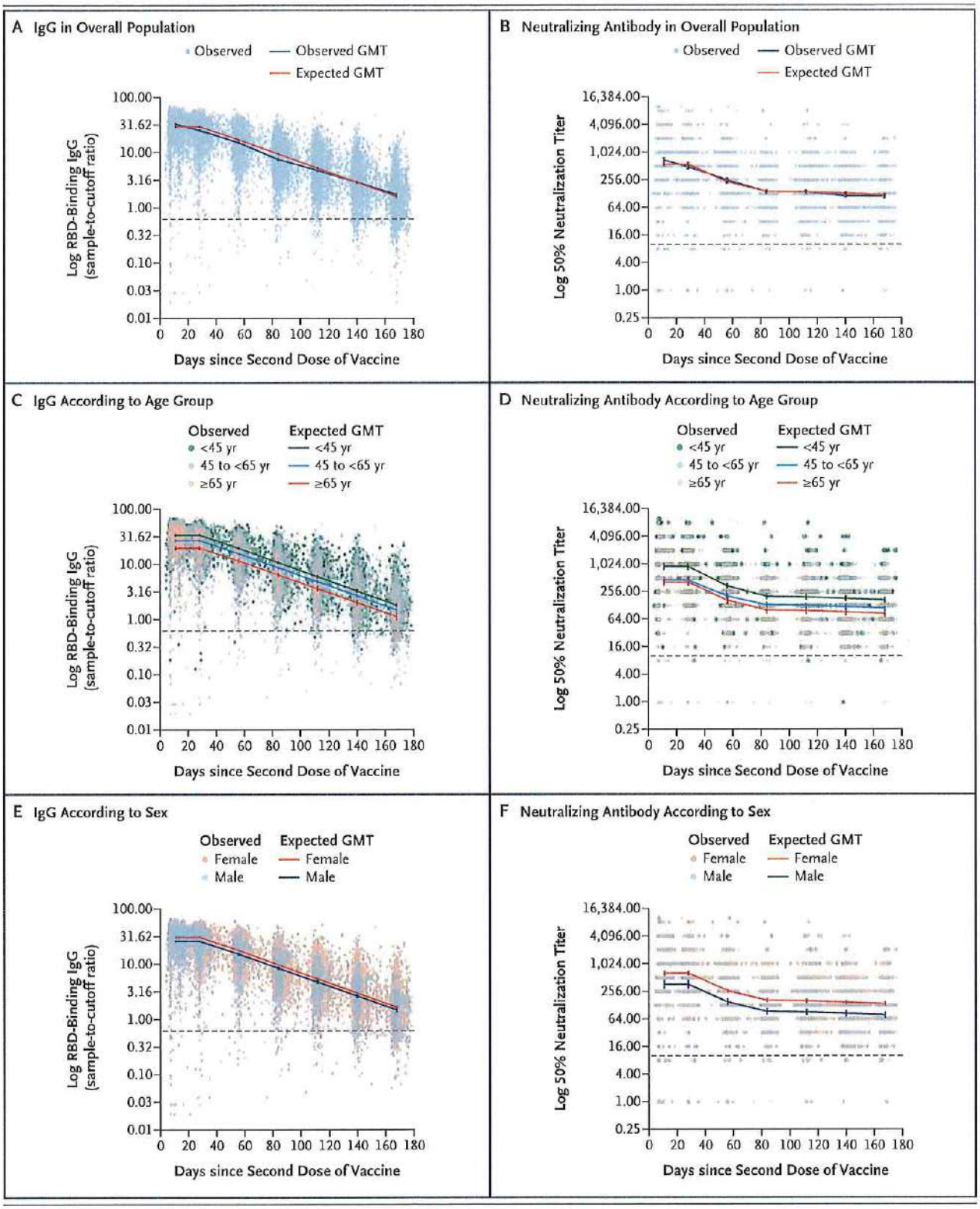

Figure 2 (facing page). Distribution of Antibodies 6 Months after Receipt of Second Dose of the BNT162b2 Vaccine.
Panels A and B show the geometric mean titers (GMTs) of IgG and neutralizing antibody, respectively, in the entire study population, and Panels C through F show GMTs according to age group and sex. Antibodies were tested monthly throughout seven periods after receipt of the second dose of vaccine. Dots represent individual observed serum samples. The dashed line in each panel indicates the cutoff for diagnostic positivity. I bars indicate 95% confidence intervals. RBD denotes receptor-binding domain.

IgG titer but not neutralizing antibody titer during both the peak and end-of-study periods than did those without autoimmune disease. An age-by-sex interaction was found; the difference by which the titers in men 45 years of age or older were lower than the titers in men younger than 45 years of age was larger than the difference between the corresponding female groups.

At the end of study, the mixed-model analysis showed decreases in IgG and neutralizing antibody concentrations of 38% and 42%, respectively, among persons 65 years of age or older as compared with participants 18 to less than 45 years of age and of 37% and 46%, respectively, among men 65 years of age or older as compared with women in the same age group (Table 1). Participants with immunosuppression had decreases in the IgG and neutralizing antibody concentrations of 65% and 70%, respectively, as compared with participants without immunosuppression. Obese participants (those with a BMI of ≥30) had a 31% increase in neutralizing antibody concentrations as compared with nonobese participants (Table 1).

For IgG levels, the correlation between individual participants' peak levels and their slopes of the decrease was positive but weak (0.17; 95% CI, 0.11 to 0.24); the rates of decay were not strongly related to initial levels. However, for neutralizing antibody, the correlation was strongly negative (−0.63; 95% CI, −0.70 to −0.55). After adjustment for other factors, participants with a higher initial level tended to have a decrease that was faster up to approximately 70 days after receipt of the second dose. Beyond that time, rates of decay were modest and did not vary much among participants.

We used the mixed model to predict the probability in different subgroups of reaching a neutralizing antibody titer lower than the test cutoff for diagnostic positivity (i.e., <16) by 6 months after receipt of the second dose. We also used the model to predict the probability of a decrease to below different neutralizing antibody titers (<32, <64, <128, or <256) (Table 2). Among healthy women and men in the three age groups (18 to <45 years, 45 to <65 years, and ≥65 years of age), the probability of having a neutralizing antibody titer of less than 256 at 175 days after receipt of the second dose were as follows: 0.68, 0.79, and 0.81, respectively, among women and 0.75, 0.89, and 0.92, respectively, among men. The probability of having a neutralizing antibody titer of less than 16 in these three age groups (18 to <45 years, 45 to <65 years, and ≥65 years of age) were as follows: 0.02, 0.05, and 0.06, respectively, among women and 0.04, 0.11, and 0.15, respectively, among men. Overall (regardless of sex and age group), obese participants were at lower risk for having lower neutralizing antibody titers than nonobese participants. Participants with immunosuppression were more likely than healthy participants to have a below-average neutralizing antibody titer (Table 2).

CORRELATION BETWEEN IGG AND NEUTRALIZING ANTIBODY LEVELS

We assessed the correlation between IgG and neutralizing antibody levels. Although a strong correlation between IgG and neutralizing antibody titers was maintained throughout the 6 months after receipt of the second dose of vaccine (Spearman's rank correlation between 0.68 and 0.75) (Fig. S2), the regression relationship between the IgG and neutralizing antibody levels depended on the time since the second dose of vaccine, a finding that was probably due to the different kinetics between IgG and neutralizing antibody levels (Fig. 2).

DISCUSSION

In this prospective longitudinal study, we found a significant waning of humoral responses within 6 months after receipt of the second dose of BNT162b2 vaccine in a large cohort of 4868

Table 1. Mixed-Model Analysis of Variables Associated with IgG and Neutralizing Antibody Titers after Receipt of the Second Vaccine Dose.*

Variable	Peak Titer		End-of-Study Titer	
	IgG (N = 3808)	Neutralizing Antibody (N = 1149)	IgG (N = 3808)	Neutralizing Antibody (N = 1149)
	ratio of mean titer (95% CI)			
Age group†				
<45 yr	Reference	Reference	Reference	Reference
45 to <65 yr	0.80 (0.77–0.84)	0.52 (0.43–0.64)	0.81 (0.78–0.85)	0.66 (0.57–0.76)
≥65 yr	0.61 (0.56–0.66)	0.59 (0.47–0.75)	0.62 (0.57–0.68)	0.58 (0.48–0.70)
Sex†				
Female	Reference	Reference	Reference	Reference
Male	0.98 (0.98–0.98)	0.64 (0.52–0.80)	0.99 (0.97–1.00)	0.64 (0.55–0.75)
Coexisting condition				
Body-mass index ≥30				
No	Reference	Reference	Reference	Reference
Yes	1.05 (0.99–1.11)	1.31 (1.14–1.51)	0.99 (0.93–1.05)	1.31 (1.14–1.51)
No. of specific coexisting conditions‡				
0	Reference	Reference	Reference	Reference
1	1.02 (0.96–1.08)	0.88 (0.70–1.11)	1.02 (0.96–1.08)	0.96 (0.84–1.17)
≥2	0.82 (0.75–0.89)	0.59 (0.44–0.79)	0.82 (0.75–0.89)	0.88 (0.71–1.09)
Autoimmune disease§				
No	Reference	Reference	Reference	Reference
Yes	0.88 (0.81–0.95)	1.15 (0.94–1.39)	0.88 (0.81–0.95)	1.15 (0.94–1.39)
Immunosuppression§				
No	Reference	Reference	Reference	Reference
Yes	0.35 (0.29–0.42)	0.30 (0.20–0.46)	0.35 (0.29–0.42)	0.30 (0.20–0.46)
Interactions between age and sex				
Age <45 yr				
Female sex	Reference	Reference	Reference	Reference
Male sex	0.89 (0.84–0.95)	0.53 (0.36–0.79)	0.96 (0.89–1.03)	0.81 (0.60–1.08)
Age 45 to <65 yr				
Female sex	Reference	Reference	Reference	Reference
Male sex	0.88 (0.82–0.94)	0.79 (0.54–1.15)	0.87 (0.81–0.94)	0.62 (0.50–0.77)
Age ≥65 yr				
Female sex	Reference	Reference	Reference	Reference
Male sex	0.70 (0.61–0.80)	0.64 (0.45–0.89)	0.63 (0.54–0.73)	0.54 (0.41–0.73)

* The peak period was defined as days 4 through 30 after receipt of the second dose of vaccine, and the end of study as day 175 after receipt of the second dose.
† Shown is the marginal effect from the mixed model without the age-by-sex interaction.
‡ Specific coexisting conditions included hypertension, diabetes, dyslipidemia, heart disease, lung disease, kidney disease, and liver disease.
§ Any participant with an autoimmune disease who also received an immunosuppressive drug was also considered to have immunosuppression.

Table 2. Probability of Having a Titer below Different Neutralizing Antibody Titers at 175 Days after Receipt of the Second Vaccine Dose, According to Sex and Age.

Sex and Titer	Probability among Healthy Persons (95% CI)*			Probability among Persons with BMI ≥30 (95% CI)			Probability among Persons with Immunosuppression (95% CI)†		
	18 to <45 Yr	45 to <65 Yr	≥65 Yr	18 to <45 Yr	45 to <65 Yr	≥65 Yr	18 to <45 Yr	45 to <65 Yr	≥65 Yr
					percent				
Female sex									
<16	0.02 (0.02–0.04)	0.05 (0.04–0.07)	0.06 (0.04–0.09)	0.01 (0.01–0.02)	0.03 (0.02–0.04)	0.04 (0.02–0.06)	0.18 (0.10–0.29)	0.28 (0.17–0.41)	0.30 (0.18–0.45)
<32	0.09 (0.07–0.11)	0.15 (0.12–0.18)	0.17 (0.13–0.22)	0.06 (0.04–0.08)	0.10 (0.08–0.13)	0.12 (0.08–0.17)	0.38 (0.25–0.52)	0.51 (0.36–0.65)	0.53 (0.38–0.68)
<64	0.23 (0.19–0.27)	0.34 (0.29–0.39)	0.36 (0.30–0.44)	0.16 (0.12–0.21)	0.26 (0.21–0.31)	0.28 (0.21–0.36)	0.62 (0.47–0.75)	0.73 (0.60–0.84)	0.75 (0.62–0.86)
<128	0.45 (0.40–0.50)	0.58 (0.53–0.62)	0.60 (0.53–0.68)	0.36 (0.30–0.42)	0.48 (0.42–0.54)	0.51 (0.42–0.60)	0.82 (0.70–0.90)	0.89 (0.80–0.95)	0.90 (0.82–0.96)
<256	0.68 (0.64–0.73)	0.79 (0.75–0.83)	0.81 (0.75–0.86)	0.60 (0.53–0.66)	0.72 (0.66–0.76)	0.74 (0.66–0.80)	0.93 (0.87–0.97)	0.97 (0.93–0.99)	0.97 (0.93–0.99)
Male sex									
<16	0.04 (0.02–0.06)	0.11 (0.08–0.16)	0.15 (0.10–0.21)	0.02 (0.01–0.04)	0.08 (0.05–0.11)	0.10 (0.06–0.16)	0.24 (0.12–0.38)	0.44 (0.28–0.60)	0.50 (0.34–0.67)
<32	0.12 (0.08–0.18)	0.28 (0.21–0.34)	0.34 (0.25–0.42)	0.08 (0.05–0.13)	0.20 (0.15–0.26)	0.25 (0.18–0.34)	0.45 (0.29–0.62)	0.67 (0.52–0.81)	0.73 (0.58–0.85)
<64	0.29 (0.21–0.38)	0.50 (0.43–0.58)	0.57 (0.48–0.66)	0.22 (0.14–0.30)	0.41 (0.32–0.49)	0.48 (0.38–0.58)	0.68 (0.53–0.82)	0.85 (0.74–0.93)	0.88 (0.79–0.95)
<128	0.52 (0.42–0.62)	0.73 (0.67–0.79)	0.78 (0.71–0.85)	0.43 (0.33–0.53)	0.65 (0.57–0.72)	0.71 (0.62–0.79)	0.86 (0.75–0.94)	0.95 (0.90–0.98)	0.96 (0.92–0.99)
<256	0.75 (0.66–0.82)	0.89 (0.85–0.92)	0.92 (0.88–0.95)	0.66 (0.57–0.76)	0.84 (0.78–0.89)	0.88 (0.82–0.92)	0.95 (0.90–0.98)	0.99 (0.97–0.997)	0.99 (0.98–0.998)

* Healthy persons were defined as participants without hypertension, diabetes, dyslipidemia, heart disease, lung disease, liver disease, immunosuppression, or autoimmune disease and with a BMI of less than 30.

† Immunosuppression included organ transplantation, biologic therapy, chemotherapy, glucocorticoids, splenectomy, and human immunodeficiency virus infection.

participants. We observed a continuous decrease in anti-S IgG titers at a relative stable rate within 6 months. The decrease in neutralizing antibody titers was brisk initially, in the period of up to 70 to 80 days, but slowed thereafter. Antibody titers were associated with age, sex, and coexisting conditions. Particularly vulnerable populations with lower neutralizing titers were older men and participants with immunosuppression.

Published work about many vaccines, such as those against measles, mumps, and rubella, has shown a small decrease each year of 5 to 10% in the neutralizing antibody levels.[13,14] We found that a significant and rapid decrease in humoral response to the BNT162b2 vaccine was observed within months after vaccination.

Neutralizing antibodies have been shown to correlate with protection.[6,15] Yet, neutralizing assays are complex and time-consuming. Thus, the correlation between anti-S IgG and neutralizing antibody levels reported here are useful. Although we found a consistently strong correlation, the regression relationship between IgG and neutralizing antibody was dependent on time. Thus, relating IgG levels to neutralizing ability depends on time since the second dose.

Using a mixed model, we analyzed the association of age, sex, and coexisting conditions with immunogenicity, both at the peak and at 6 months after receipt of the second dose. We found that antibody levels in both periods were higher in women than in men and decreased with age, as has been previously shown for the first month after receipt of the second dose.[7,16] Similar to the findings in other reports,[17-19] a significantly lower antibody response was found consistently through the observation period among participants with immunosuppression, who had neutralizing antibody titers that were lower by a factor of 5 than those among participants without immunosuppression.

Obese persons (BMI, ≥30) had a significantly higher neutralizing antibody titer during long-term follow-up than nonobese participants. Obesity is associated with severe Covid-19,[20] and disease severity is associated with a higher Covid-19 humoral immune response. A recent study showed that SARS-CoV-2 neutralizing antibodies are positively associated with BMI.[21] Yet, it is still unclear whether vaccinated obese persons are at higher or lower risk for breakthrough infection and whether the relatively high humoral response to the vaccine is protective.

Several studies on the durability of humoral response in persons who have recovered from SARS-CoV-2 infection showed that both IgG and neutralizing antibody levels decrease only modestly at 8 to 10 months after the infection.[22,23] This striking difference in antibody kinetics between convalescent persons and vaccinated persons may be the reason for the substantially lower incidence of breakthrough infection among previously infected persons than among vaccinated persons.[24,25] Overall, the accumulating evidence from our study and others[22-25] shows that long-term humoral response and vaccine effectiveness in previously infected persons were superior to that in recipients of two doses of vaccine.

Our study was conducted in a cohort of health care workers, who were mostly healthy persons and therefore may not represent the general population. To overcome this limitation, although IgG tests were performed in the entire study population, neutralizing antibody tests were performed in a subgroup that included higher proportions of older persons or of persons with coexisting conditions in order to better represent the general population.

Our data provide important insights into the longitudinal dynamics of the immune response to BNT162b2 vaccination. As this pandemic continues to evolve, the importance of determining immune correlates of protection after vaccination becomes clearer. Strategies to prolong host immunity need to be evaluated in order to protect the population against SARS-CoV-2 and its variants.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention (CDC)
Atlanta GA 30333

November 05, 2021

SENT VIA EMAIL

Elizabeth Brehm
Attorney
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New York, New York 10166
foia@sirillp.com

2nd Letter Subject: Final Response Letter

Dear Ms. Brehm:

The Centers for Disease Control and Prevention and Agency for Toxic Substances and Disease Registry (CDC/ATSDR) received your September 02, 2021, Freedom of Information Act (FOIA) request on September 02, 2021, seeking:

> "Documents reflecting any documented case of an individual who: (1) never received a COVID-19 vaccine; (2) was infected with COVID-19 once, recovered, and then later became infected again; and (3) transmitted SARS-CoV-2 to another person when reinfected."

A search of our records failed to reveal any documents pertaining to your request. The CDC Emergency Operations Center (EOC) conveyed that this information is not collected.

You may contact our FOIA Public Liaison at 770-488-6277 for any further assistance and to discuss any aspect of your request. Additionally, you may contact the Office of Government Information Services (OGIS) at the National Archives and Records Administration to inquire about the FOIA mediation services they offer. The contact information for OGIS is as follows: Office of Government Information Services, National Archives and Records Administration, 8601 Adelphi Road-OGIS, College Park, Maryland 20740-6001, e-mail at ogis@nara.gov; telephone at 202-741-5770; toll free at 1-877-684-6448; or facsimile at 202-741-5769.

If you are not satisfied with the response to this request, you may administratively appeal by writing to the Deputy Agency Chief FOIA Officer, Office of the Assistant Secretary for Public Affairs, U.S. Department of Health and Human Services, Hubert H. Humphrey Building, 200 Independence Avenue, Suite 729H, Washington, D.C. 20201. You may also transmit your appeal via email to FOIARequest@psc.hhs.gov. Please mark both your appeal letter and envelope "FOIA Appeal." Your appeal must be postmarked or electronically transmitted by February 03, 2022.

Sincerely,

Roger Andoh
CDC/ATSDR FOIA Officer
Office of the Chief Operating Officer
Phone: (770) 488-6399
Fax: (404) 235-1852

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Article

SARS-CoV-2 infection induces long-lived bone marrow plasma cells in humans

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Long-lived bone marrow plasma cells (BMPCs) are a persistent and essential source of protective antibodies[1–7]. Individuals who have recovered from COVID-19 have a substantially lower risk of reinfection with SARS-CoV-2[8–10]. Nonetheless, it has been reported that levels of anti-SARS-CoV-2 serum antibodies decrease rapidly in the first few months after infection, raising concerns that long-lived BMPCs may not be generated and humoral immunity against SARS-CoV-2 may be short-lived[11–13]. Here we show that in convalescent individuals who had experienced mild SARS-CoV-2 infections (n = 77), levels of serum anti-SARS-CoV-2 spike protein (S) antibodies declined rapidly in the first 4 months after infection and then more gradually over the following 7 months, remaining detectable at least 11 months after infection. Anti-S antibody titres correlated with the frequency of S-specific plasma cells in bone marrow aspirates from 18 individuals who had recovered from COVID-19 at 7 to 8 months after infection. S-specific BMPCs were not detected in aspirates from 11 healthy individuals with no history of SARS-CoV-2 infection. We show that S-binding BMPCs are quiescent, which suggests that they are part of a stable compartment. Consistently, circulating resting memory B cells directed against SARS-CoV-2 S were detected in the convalescent individuals. Overall, our results indicate that mild infection with SARS-CoV-2 induces robust antigen-specific, long-lived humoral immune memory in humans.

Reinfections by seasonal coronaviruses occur 6 to 12 months after the previous infection, indicating that protective immunity against these viruses may be short-lived[14,15]. Early reports documenting rapidly declining antibody titres in the first few months after infection in individuals who had recovered from COVID-19 suggested that protective immunity against SARS-CoV-2 might be similarly transient[11–13]. It was also suggested that infection with SARS-CoV-2 could fail to elicit a functional germinal centre response, which would interfere with the generation of long-lived plasma cells[3–5,7,16]. More recent reports analysing samples that were collected approximately 4 to 6 months after infection indicate that SARS-CoV-2 antibody titres decline more slowly than in the initial months after infection[8,17–21]. Durable serum antibody titres are maintained by long-lived plasma cells—non-replicating, antigen-specific plasma cells that are detected in the bone marrow long after the clearance of the antigen[1–7]. We sought to determine whether they were detectable in convalescent individuals approximately 7 months after SARS-CoV-2 infection.

Biphasic decay of anti-S antibody titres

Blood samples were collected approximately 1 month after the onset of symptoms from 77 individuals who were convalescing from COVID-19 (49% female, 51% male, median age 49 years), the majority of whom had experienced mild illness (7.8% hospitalized, Extended Data Tables 1, 2). Follow-up blood samples were collected three times at approximately three-month intervals. Twelve convalescent participants received either the BNT162b2 (Pfizer) or the mRNA-1273 (Moderna) SARS-CoV-2 vaccine between the last two time points; these post-vaccination samples were not included in our analyses. In addition, bone marrow aspirates were collected from 18 of the convalescent individuals at 7 to 8 months after infection and from 11 healthy volunteers with no history of SARS-CoV-2 infection or vaccination. Follow-up bone marrow aspirates were collected from 5 of the 18 convalescent individuals and from 1 additional convalescent donor approximately 11 months after infection (Fig. 1a, Extended Data Tables 3, 4). We first performed a longitudinal analysis of circulating anti-SARS-CoV-2 serum antibodies. Whereas anti-SARS-CoV-2 spike protein (S) IgG antibodies were undetectable in blood from control individuals, 74 out of the 77 convalescent individuals had detectable serum titres approximately 1 month after the onset of symptoms. Between 1 and 4 months after symptom onset, overall anti-S IgG titres decreased from a mean $\log_e$-transformed half-maximal dilution of 6.3 to 5.7 (mean difference 0.59 ± 0.06, P < 0.001). However, in the interval between 4 and 11 months after symptom onset, the rate

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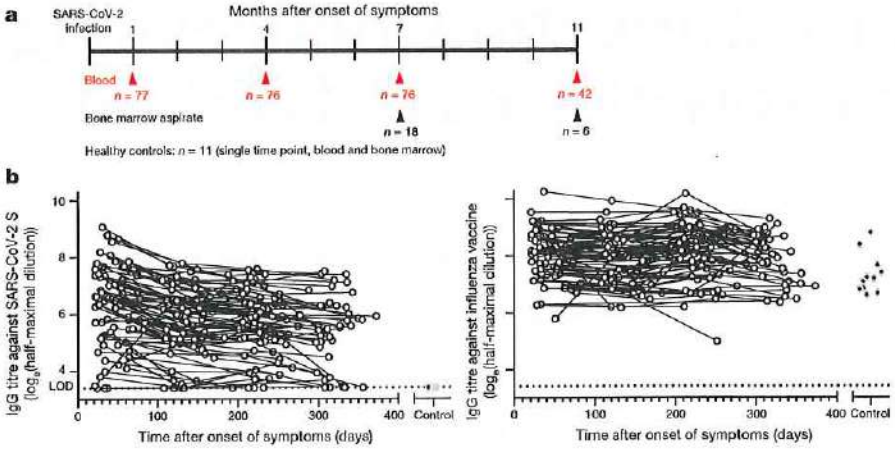

Fig. 1 | SARS-CoV-2 infection elicits durable serum anti-S antibody titres. **a**, Study design. Seventy-seven convalescent individuals who had experienced mild SARS-CoV-2 infections (aged 21–69 years) were enrolled and blood was collected approximately 1 month, 4 months, 7 months and 11 months after the onset of symptoms. Bone marrow aspirates were collected from 18 of the convalescent individuals 7 to 8 months after infection and from 11 healthy volunteers (aged 23–60 years) with no history of SARS-CoV-2 infection. Follow-up bone marrow aspirates were collected from 5 of the 18 convalescent donors and 1 additional convalescent donor approximately 11 months after infection. **b**, Blood IgG titres against SARS-CoV-2 S (left) and influenza virus vaccine (right) measured by enzyme-linked immunosorbent assay (ELISA) in convalescent individuals (white circles) at the indicated time after onset of symptoms, and in control individuals (black circles). The dotted lines indicate the limit of detection (LOD). Mean titres and pairwise differences at each time point were estimated using a linear mixed model analysis.

of decline slowed, and mean titres decreased from 5.7 to 5.3 (mean difference 0.44 ± 0.10, $P < 0.001$; Fig. 1a). In contrast to the anti-S antibody titres, IgG titres against the 2019–2020 inactivated seasonal influenza virus vaccine were detected in all control individuals and individuals who were convalescing from COVID-19, and declined much more gradually, if at all over the course of the study, with mean titres decreasing from 8.0 to 7.9 (mean difference 0.16 ± 0.06, $P = 0.042$) and 7.9 to 7.8 (mean difference 0.02 ± 0.08, $P = 0.997$) across the 1-to-4-month and 4-to-11-month intervals after symptom onset, respectively (Fig. 1b).

Induction of S-binding long-lived BMPCs

The relatively rapid early decline in the levels of anti-S IgG, followed by a slower decrease, is consistent with a transition from serum antibodies being secreted by short-lived plasmablasts to secretion by a smaller but more persistent population of long-lived plasma cells generated later in the immune response. The majority of this latter population resides in the bone marrow[1–6]. To investigate whether individuals who had recovered from COVID-19 developed a virus-specific long-lived BMPC compartment, we examined bone marrow aspirates obtained approximately 7 and 11 months after infection for anti-SARS-CoV-2 S-specific BMPCs. We magnetically enriched BMPCs from the aspirates and then quantified the frequencies of those secreting IgG and IgA directed against the 2019–2020 influenza virus vaccine, the tetanus–diphtheria vaccine and SARS-CoV-2 S by enzyme-linked immunosorbent spot assay (ELISpot) (Fig. 2a). Frequencies of influenza- and tetanus–diphtheria-vaccine-specific BMPCs were comparable between control individuals and convalescent individuals. IgG- and IgA-secreting S-specific BMPCs were detected in 15 and 9 of the 19 convalescent individuals, respectively, but not in any of the 11 control individuals (Fig. 2b). Notably, none of the control individuals or convalescent individuals had detectable S-specific antibody-secreting cells in the blood at the time of bone marrow sampling, indicating that the detected BMPCs represent bone-marrow-resident cells and not contamination from circulating plasmablasts. Frequencies of anti-S IgG BMPCs were stable among the 5 convalescent individuals who were sampled a second time approximately 4 months later, and frequencies of anti-S IgA BMPCs were stable in 4 of these 5 individuals but had decreased to below the limit of detection in one individual (Fig. 2c). Consistent with their stable BMPC frequencies, anti-S IgG titres in the 5 convalescent individuals remained consistent between 7 and 11 months after symptom onset. IgG titres measured against the receptor-binding domain (RBD) of the S protein—a primary target of neutralizing antibodies—were detected in 4 of the 5 convalescent individuals and were also stable between 7 and 11 months after symptom onset (Fig. 2d). Frequencies of anti-S IgG BMPCs showed a modest but significant correlation with circulating anti-S IgG titres at 7–8 months after the onset of symptoms in convalescent individuals, consistent with the long-term maintenance of antibody levels by these cells ($r = 0.48$, $P = 0.046$). In accordance with previous reports[22–24], frequencies of influenza-vaccine-specific IgG BMPCs and antibody titres exhibited a strong and significant correlation ($r = 0.67$, $P < 0.001$; Fig. 2e). Nine of the aspirates from control individuals and 12 of the 18 aspirates that were collected 7 months after symptom onset from convalescent individuals yielded a sufficient number of BMPCs for additional analysis by flow cytometry. We stained these samples intracellularly with fluorescently labelled S and influenza virus haemagglutinin (HA) probes to identify and characterize antigen-specific BMPCs. As controls, we also intracellularly stained peripheral blood mononuclear cells (PBMCs) from healthy volunteers one week after vaccination against SARS-CoV-2 or seasonal influenza virus (Fig. 3a, Extended Data Fig. 1a–c). Consistent with the ELISpot data, low frequencies of S-binding BMPCs were detected in 10 of the 12 samples from convalescent individuals, but not in any of the 9 control samples (Fig. 3b). Although both recently generated circulating plasmablasts and S- and HA-binding BMPCs expressed BLIMP-1, the BMPCs were differentiated by their lack of expression of Ki-67—indicating a quiescent state—as well as by higher levels of CD38 (Fig. 3c).

Robust S-binding memory B cell response

Memory B cells form the second arm of humoral immune memory. After re-exposure to an antigen, memory B cells rapidly expand and differentiate into antibody-secreting plasmablasts. We examined the frequency of SARS-CoV-2-specific circulating memory B cells in individuals who were convalescing from COVID-19 and in healthy control individuals. We stained PBMCs with fluorescently labelled S probes and determined the frequency of S-binding memory B cells among isotype-switched $IgD^{lo}CD20^{+}$ memory B cells by flow cytometry. For comparison, we

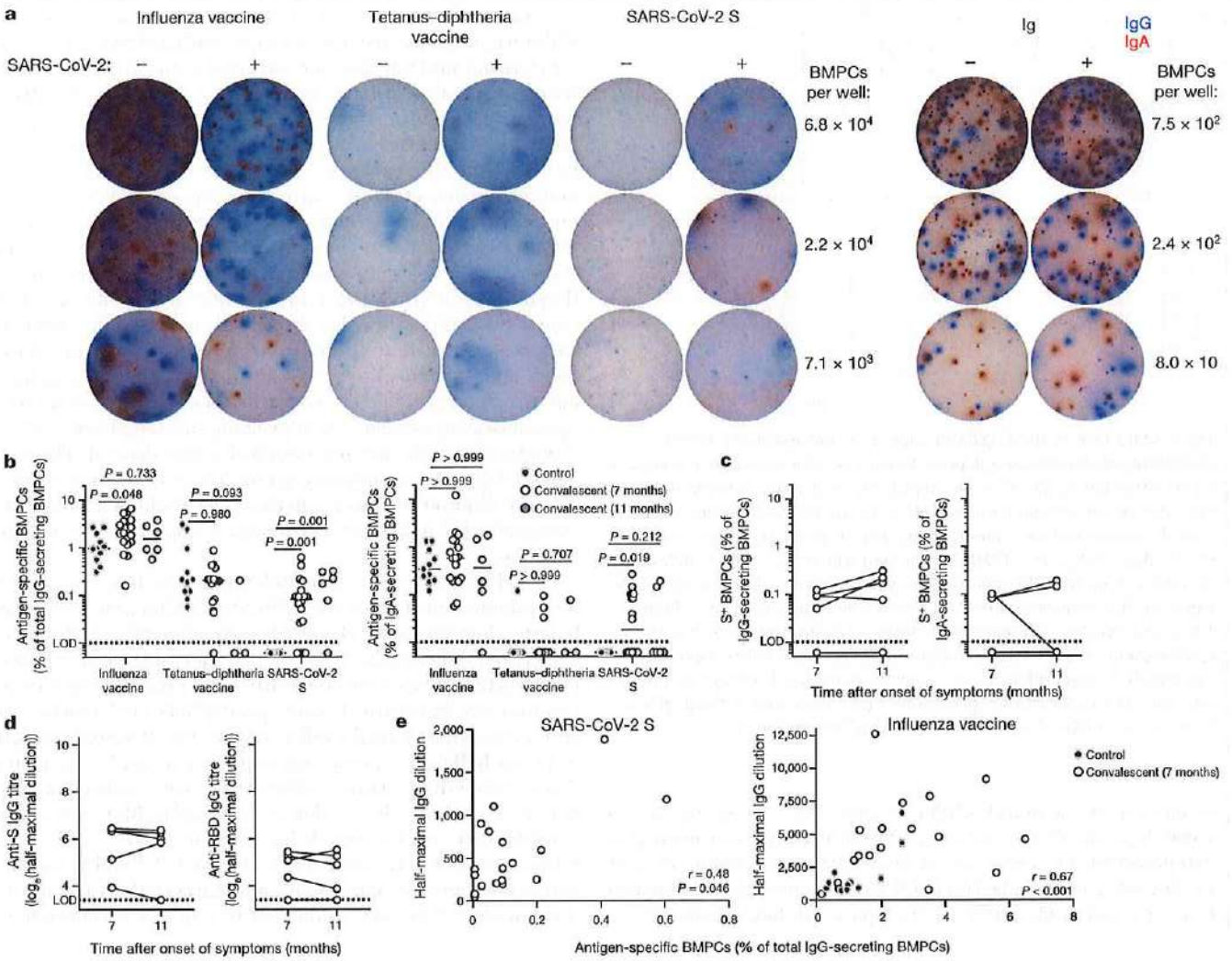

Fig. 2 | SARS-CoV-2 infection elicits S-binding long-lived BMPCs. **a**, Representative images of ELISpot wells coated with the indicated antigens or anti-immunoglobulin (Ig) and developed in blue and red for IgG and IgA, respectively, after incubation of magnetically enriched BMPCs from control individuals and convalescent individuals. **b**, Frequencies of BMPCs secreting IgG (left) or IgA (right) antibodies specific for the indicated antigens, indicated as percentages of total IgG- or IgA-secreting BMPCs in control individuals (black circles) or convalescent individuals 7 months (white circles) or 11 months (grey circles) after symptom onset. Horizontal lines indicate the median. *P* values from two-sided Kruskal–Wallis tests with Dunn's correction for multiple comparisons between control individuals and convalescent individuals. Each symbol represents one sample ($n = 18$ convalescent, $n = 11$ control). **c**, Paired frequencies of S-binding BMPCs among IgG-secreting (left) and IgA-secreting (right) BMPCs from convalescent individuals 7 months and 11 months after symptom onset. **d**, Paired anti-S (left) and anti-RBD (right) IgG serum antibody titres from convalescent individuals 7 months and 11 months after symptom onset. Data in **c** and **d** (left) are also shown in **b** and Fig. 1b, respectively. Each symbol represents one sample ($n = 5$). Dotted lines indicate the limit of detection. **e**, Frequencies of BMPCs secreting IgG antibodies specific for SARS-CoV-2 S (left) and influenza virus vaccine (right) plotted against respective IgG titres in paired blood samples from control individuals (black circles) or convalescent individuals 7 months after symptom onset (white circles). *P* and *r* values from two-sided Spearman's correlations. Each symbol represents one sample ($n = 18$ convalescent, $n = 11$ control).

co-stained the cells with fluorescently labelled influenza virus HA probes (Fig. 4a, Extended Data Fig. 1d). S-binding memory B cells were identified in convalescent individuals in the first sample that was collected approximately one month after the onset of symptoms, with comparable frequencies to influenza HA-binding memory B cells (Fig. 4b). S-binding memory B cells were maintained for at least 7 months after symptom onset and were present at significantly higher frequencies relative to healthy controls—comparable to the frequencies of influenza HA-binding memory B cells that were identified in both groups (Fig. 4c).

Discussion

This study sought to determine whether infection with SARS-CoV-2 induces antigen-specific long-lived BMPCs in humans. We detected SARS-CoV-2 S-specific BMPCs in bone marrow aspirates from 15 out of 19 convalescent individuals, and in none from the 11 control participants. The frequencies of anti-S IgG BMPCs modestly correlated with serum IgG titres at 7–8 months after infection. Phenotypic analysis by flow cytometry showed that S-binding BMPCs were quiescent, and their frequencies were largely consistent in 5 paired aspirates collected at 7 and 11 months after symptom onset. Notably, we detected no S-binding cells among plasmablasts in blood samples collected at the same time as the bone marrow aspirates by ELISpot or flow cytometry in any of the convalescent or control samples. Together, these data indicate that mild SARS-CoV-2 infection induces a long-lived BMPC response. In addition, we showed that S-binding memory B cells in the blood of individuals who had recovered from COVID-19 were present at similar frequencies to those directed against influenza virus HA. Overall, our

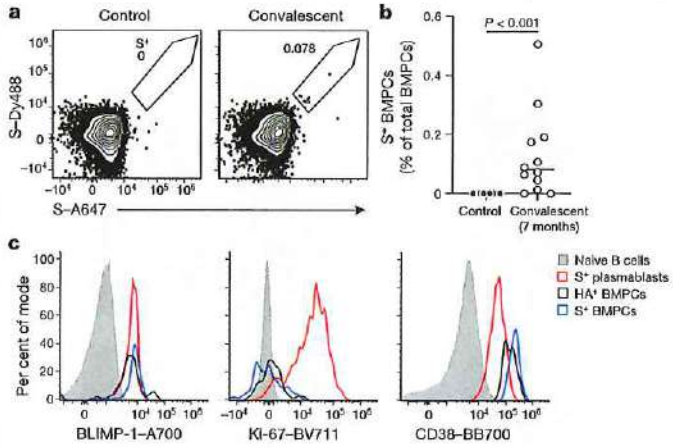

Fig. 3 | SARS-CoV-2 S-binding BMPCs are quiescent and distinct from circulating plasmablasts. **a**, Representative plots of intracellular S staining in $CD20^{lo}CD38^{+}IgD^{lo}CD19^{+/lo}CD3^{-}$ live singlet BMPCs (gating in Extended Data Fig. 1a) from magnetically enriched BMPCs from control individuals (left) or convalescent individuals 7 months after symptom onset (right). **b**, Frequencies of S-binding BMPCs in total BMPCs from control individuals (black circles) or convalescent individuals 7 months after symptom onset (white circles). Horizontal lines indicate the median. *P* value from two-sided Mann–Whitney *U* test. Each symbol represents one sample ($n = 12$ convalescent, $n = 9$ control). **c**, Histograms of BLIMP-1 (left), Ki-67 (centre), and CD38 (right) staining in S^{+} (blue) and HA^{+} (black) BMPCs from magnetically enriched BMPCs 7 months after symptom onset, and in S^{+} plasmablasts (red) and naive B cells (grey) from healthy donor PBMCs 1 week after SARS-CoV-2 S immunization.

results are consistent with SARS-CoV-2 infection eliciting a canonical T-cell-dependent B cell response, in which an early transient burst of extrafollicular plasmablasts generates a wave of serum antibodies that decline relatively quickly. This is followed by more stably maintained levels of serum antibodies that are supported by long-lived BMPCs.

Although this overall trend captures the serum antibody dynamics of the majority of participants, we observed that in three participants, anti-S serum antibody titres increased between 4 and 7 months after the onset of symptoms, after having initially declined between 1 and 4 months. This could be stochastic noise, could represent increased net binding affinity as early plasmablast-derived antibodies are replaced by those from affinity-matured BMPCs, or could represent increases in antibody concentration from re-encounter with the virus (although none of the participants in our cohort tested positive a second time). Although anti-S IgG titres in the convalescent cohort were relatively stable in the interval between 4 and 11 months after symptom onset, they did measurably decrease, in contrast to anti-influenza virus vaccine titres. It is possible that this decline reflects a final waning of early plasmablast-derived antibodies. It is also possible that the lack of decline in influenza titres was due to boosting through exposure to influenza antigens. Our data suggest that SARS-CoV-2 infection induces a germinal centre response in humans because long-lived BMPCs are thought to be predominantly germinal-centre-derived[7]. This is consistent with a recent study that reported increased levels of somatic hypermutation in memory B cells that target the RBD of SARS-CoV-2 S in convalescent individuals at 6 months compared to 1 month after infection[20].

To our knowledge, the current study provides the first direct evidence for the induction of antigen-specific BMPCs after a viral infection in humans. However, we do acknowledge several limitations. Although we detected anti-S IgG antibodies in serum at least 7 months after infection in all 19 of the convalescent donors from whom we obtained bone marrow aspirates, we failed to detect S-specific BMPCs in 4 donors. Serum anti-S antibody titres in those four donors were low, suggesting that S-specific BMPCs may potentially be present at very low frequencies that are below the limit of detection of the assay. Another limitation is that we do not know the fraction of the S-binding BMPCs detected in our study that encodes neutralizing antibodies. SARS-CoV-2 S protein is the main target of neutralizing antibodies[17,25–30] and a correlation between serum anti-S IgG binding and neutralization titres has been documented[17,31]. Further studies will be required to determine the

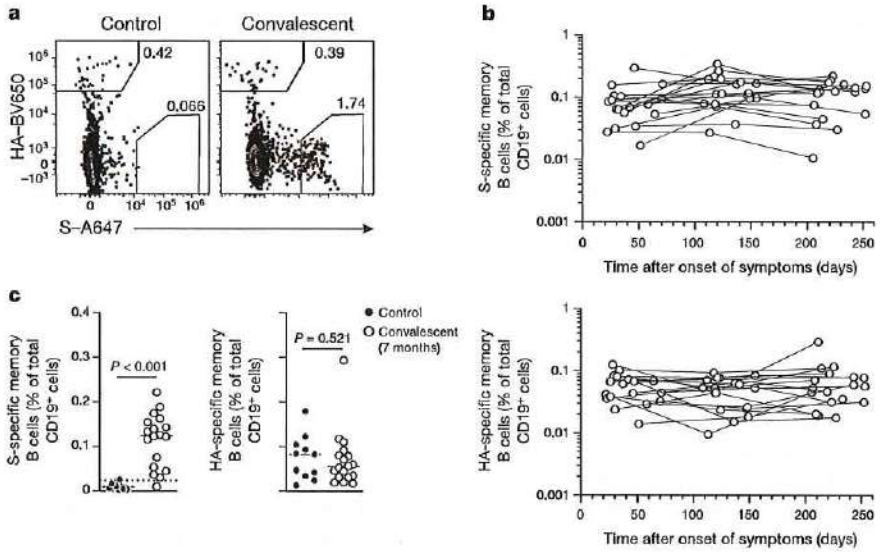

Fig. 4 | SARS-CoV-2 infection elicits a robust memory B cell response. **a**, Representative plots of surface influenza virus HA and S staining in $CD20^{+}CD38^{lo/int}IgD^{lo}CD19^{+}CD3^{-}$ live singlet memory B cells (gating in Extended Data Fig. 1d) from PBMCs from control individuals (left) and convalescent individuals 7 months after symptom onset (right). **b**, Kinetics of S- (top) and HA- (bottom) binding memory B cells in PBMCs from convalescent individuals, collected at the indicated days after symptom onset. Data from the 7-month time point are also shown in **c**. **c**, Frequencies of S- (left) and HA- (right) binding memory B cells in PBMCs from control individuals (black circles) and convalescent individuals 7 months after symptom onset (white circles). The dotted line in the left plot indicates the limit of sensitivity, which was defined as the median + 2× s.d. of the controls. Each symbol represents one sample ($n = 18$ convalescent, $n = 11$ control). Horizontal lines indicate the median. *P* values from two-sided Mann–Whitney *U* tests.

epitopes that are targeted by BMPCs and memory B cells, as well as their clonal relatedness. Finally, although our data document a robust induction of long-lived BMPCs after infection with SARS-CoV-2, it is critical to note that our convalescent individuals mostly experienced mild infections. Our data are consistent with a report showing that individuals who recovered rapidly from symptomatic SARS-CoV-2 infection generated a robust humoral immune response[32]. It is possible that more-severe SARS-CoV-2 infections could lead to a different outcome with respect to long-lived BMPC frequencies, owing to dysregulated humoral immune responses. This, however, has not been the case in survivors of the 2014 Ebola virus outbreak in West Africa, in whom severe viral infection induced long-lasting antigen-specific serum IgG antibodies[33].

Long-lived BMPCs provide the host with a persistent source of preformed protective antibodies and are therefore needed to maintain durable immune protection. However, the longevity of serum anti-S IgG antibodies is not the only determinant of how durable immune-mediated protection will be. Isotype-switched memory B cells can rapidly differentiate into antibody-secreting cells after re-exposure to a pathogen, offering a second line of defence[34]. Encouragingly, the frequency of S-binding circulating memory B cells at 7 months after infection was similar to that of B cells directed against contemporary influenza HA antigens. Overall, our data provide strong evidence that SARS-CoV-2 infection in humans robustly establishes the two arms of humoral immune memory: long-lived BMPCs and memory B cells. These findings provide an immunogenicity benchmark for SARS-CoV-2 vaccines and a foundation for assessing the durability of primary humoral immune responses that are induced in humans after viral infections.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-021-03647-4.

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Methods

Data reporting

No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded during outcome assessment.

Sample collection, preparation and storage

All studies were approved by the Institutional Review Board of Washington University in St Louis. Written consent was obtained from all participants. Seventy-seven participants who had recovered from SARS-CoV-2 infection and eleven control individuals without a history of SARS-CoV-2 infection were enrolled (Extended Data Tables 1, 4). Blood samples were collected in EDTA tubes and PBMCs were enriched by density gradient centrifugation over Ficoll 1077 (GE) or Lymphopure (BioLegend). The remaining red blood cells were lysed with ammonium chloride lysis buffer, and cells were immediately used or cryopreserved in 10% dimethyl sulfoxide in fetal bovine serum (FBS). Bone marrow aspirates of approximately 30 ml were collected in EDTA tubes from the iliac crest of 18 individuals who had recovered from COVID-19 and the control individuals. Bone marrow mononuclear cells were enriched by density gradient centrifugation over Ficoll 1077, and the remaining red blood cells were lysed with ammonium chloride buffer (Lonza) and washed with phosphate-buffered saline (PBS) supplemented with 2% FBS and 2 mM EDTA. Bone marrow plasma cells were enriched from bone marrow mononuclear cells using the CD138 Positive Selection Kit II (Stemcell) and immediately used for ELISpot or cryopreserved in 10% dimethyl sulfoxide in FBS.

Antigens

Recombinant soluble spike protein (S) and its receptor-binding domain (RBD) derived from SARS-CoV-2 were expressed as previously described[35]. In brief, mammalian cell codon-optimized nucleotide sequences coding for the soluble version of S (GenBank: MN908947.3, amino acids (aa) 1–1,213) including a C-terminal thrombin cleavage site, T4 foldon trimerization domain and hexahistidine tag cloned into the mammalian expression vector pCAGGS. The S protein sequence was modified to remove the polybasic cleavage site (RRAR to A) and two stabilizing mutations were introduced (K986P and V987P, wild-type numbering). The RBD, along with the signal peptide (aa 1–14) plus a hexahistidine tag were cloned into the mammalian expression vector pCAGGS. Recombinant proteins were produced in Expi293F cells (Thermo Fisher Scientific) by transfection with purified DNA using the ExpiFectamine 293 Transfection Kit (Thermo Fisher Scientific). Supernatants from transfected cells were collected 3 (for S) or 4 (for RBD) days after transfection, and recombinant proteins were purified using Ni-NTA agarose (Thermo Fisher Scientific), then buffer-exchanged into PBS and concentrated using Amicon Ultracel centrifugal filters (EMD Millipore). For flow cytometry staining, recombinant S was labelled with Alexa Fluor 647- or DyLight 488-NHS ester (Thermo Fisher Scientific); excess Alexa Fluor 647 and DyLight 488 were removed using 7-kDa and 40-kDa Zeba desalting columns, respectively (Pierce). Recombinant HA from A/Michigan/45/2015 (aa 18–529, Immune Technology) was labelled with DyLight 405-NHS ester (Thermo Fisher Scientific); excess DyLight 405 was removed using 7-kDa Zeba desalting columns. Recombinant HA from A/Brisbane/02/2018 (aa 18–529) and B/Colorado/06/2017 (aa 18–546) (both Immune Technology) were biotinylated using the EZ-Link Micro NHS-PEG4-Biotinylation Kit (Thermo Fisher Scientific); excess biotin was removed using 7-kDa Zeba desalting columns.

ELISpot

Plates were coated with Flucelvax Quadrivalent 2019/2020 seasonal influenza virus vaccine (Sequiris), tetanus–diphtheria vaccine (Grifols), recombinant S or anti-human Ig. Direct ex vivo ELISpot was performed to determine the number of total, vaccine-binding or recombinant S-binding IgG- and IgA-secreting cells present in BMPC and PBMC samples using IgG/IgA double-colour ELISpot Kits (Cellular Technology) according to the manufacturer's instructions. ELISpot plates were analysed using an ELISpot counter (Cellular Technology).

ELISA

Assays were performed in 96-well plates (MaxiSorp, Thermo Fisher Scientific) coated with 100 µl of Flucelvax 2019/2020 or recombinant S in PBS, and plates were incubated at 4 °C overnight. Plates were then blocked with 10% FBS and 0.05% Tween-20 in PBS. Serum or plasma were serially diluted in blocking buffer and added to the plates. Plates were incubated for 90 min at room temperature and then washed 3 times with 0.05% Tween-20 in PBS. Goat anti-human IgG–HRP (Jackson ImmunoResearch, 1:2,500) was diluted in blocking buffer before adding to wells and incubating for 60 min at room temperature. Plates were washed 3 times with 0.05% Tween-20 in PBS, and then washed 3 times with PBS before the addition of *o*-phenylenediamine dihydrochloride peroxidase substrate (Sigma-Aldrich). Reactions were stopped by the addition of 1 M HCl. Optical density measurements were taken at 490 nm. The half-maximal binding dilution for each serum or plasma sample was calculated using nonlinear regression (GraphPad Prism v.8). The limit of detection was defined as 1:30.

Statistics

Spearman's correlation coefficients were estimated to assess the relationship between 7-month anti-S and anti-influenza virus vaccine IgG titres and the frequencies of BMPCs secreting IgG specific for S and for influenza virus vaccine, respectively. Means and pairwise differences of antibody titres at each time point were estimated using a linear mixed model analysis with a first-order autoregressive covariance structure. Time since symptom onset was treated as a categorical fixed effect for the 4 different sample time points spaced approximately 3 months apart. *P* values were adjusted for multiple comparisons using Tukey's method. All analyses were conducted using SAS v.9.4 (SAS Institute) and Prism v.8.4 (GraphPad), and *P* values of less than 0.05 were considered significant.

Flow cytometry

Staining for flow cytometry analysis was performed using cryo-preserved magnetically enriched BMPCs and cryo-preserved PBMCs. For BMPC staining, cells were stained for 30 min on ice with CD45-A532 (HI30, Thermo Fisher Scientific, 1:50), CD38-BB700 (HIT2, BD Horizon, 1:500), CD19-PE (HIB19, 1:200), CXCR5-PE-Dazzle 594 (J252D4, 1:50), CD71-PE-Cy7 (CY1G4, 1:400), CD20-APC-Fire750 (2H7, 1:400), CD3-APC-Fire810 (SK7, 1:50) and Zombie Aqua (all BioLegend) diluted in Brilliant Stain buffer (BD Horizon). Cells were washed twice with 2% FBS and 2 mM EDTA in PBS (P2), fixed for 1 h using the True Nuclear permeabilization kit (BioLegend), washed twice with perm/wash buffer, stained for 1h with DyLight 405-conjugated recombinant HA from A/Michigan/45/2015, DyLight 488- and Alexa 647-conjugated S, Ki-67-BV711 (Ki-67, 1:200, BioLegend) and BLIMP-1-A700 (646702, 1:50, R&D), washed twice with perm/wash buffer, and resuspended in P2. For memory B cell staining, PBMCs were stained for 30 min on ice with biotinylated recombinant HAs diluted in P2, washed twice, then stained for 30 min on ice with Alexa 647-conjugated S, IgA-FITC (M24A, Millipore, 1:500), IgG-BV480 (goat polyclonal, Jackson ImmunoResearch, 1:100), IgD-SB702 (IA6-2, Thermo Fisher Scientific, 1:50), CD38-BB700 (HIT2, BD Horizon, 1:500), CD20-Pacific Blue (2H7, 1:400), CD4-BV570 (OKT4, 1:50), CD24-BV605 (ML5, 1:100), streptavidin-BV650, CD19-BV750 (HIB19, 1:100), CD71-PE (CY1G4, 1:400), CXCR5-PE-Dazzle 594 (J252D4, 1:50), CD27-PE-Cy7 (O323, 1:200), IgM-APC-Fire750 (MHM-88, 1:100), CD3-APC-Fire810 (SK7, 1:50) and Zombie NIR (all BioLegend) diluted in Brilliant Stain buffer (BD Horizon), and washed twice with P2. Cells were acquired on an Aurora using SpectroFlo v.2.2 (Cytek). Flow cytometry data were analysed using FlowJo v.10 (Treestar). In each experiment,

PBMCs were included from convalescent individuals and control individuals.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Relevant data are available from the corresponding author upon reasonable request.

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Author contributions A.H.E. conceived and designed the study. J.S.T. and A.H.E. designed experiments and composed the manuscript. A.H., M.K.K., I.P., J.A.O. and R.M.P. wrote and maintained the Institutional Review Board protocol, recruited and phlebotomized participants and coordinated sample collection. J.S.T., W.K., E.K., A.J.S. and L.H. processed specimens. A.J.S. expressed S and RBD proteins. J.S.T., W.K. and E.K. performed ELISA and ELISpot. J.S.T. performed flow cytometry. J.S.T., A.M.R., C.W.G. and A.H.E. analysed data. All authors reviewed the manuscript.

Competing interests The Ellebedy laboratory received funding under sponsored research agreements that are unrelated to the data presented in the current study from Emergent BioSolutions and from AbbVie. J.S.T., A.J.S. and A.H.E. are recipients of a licensing agreement with Abbvie that is unrelated to the data presented in the current study. A.H.E. is a consultant for Mubadala Investment Company and the founder of ImmuneBio Consulting. All other authors declare no competing interests.

Additional information
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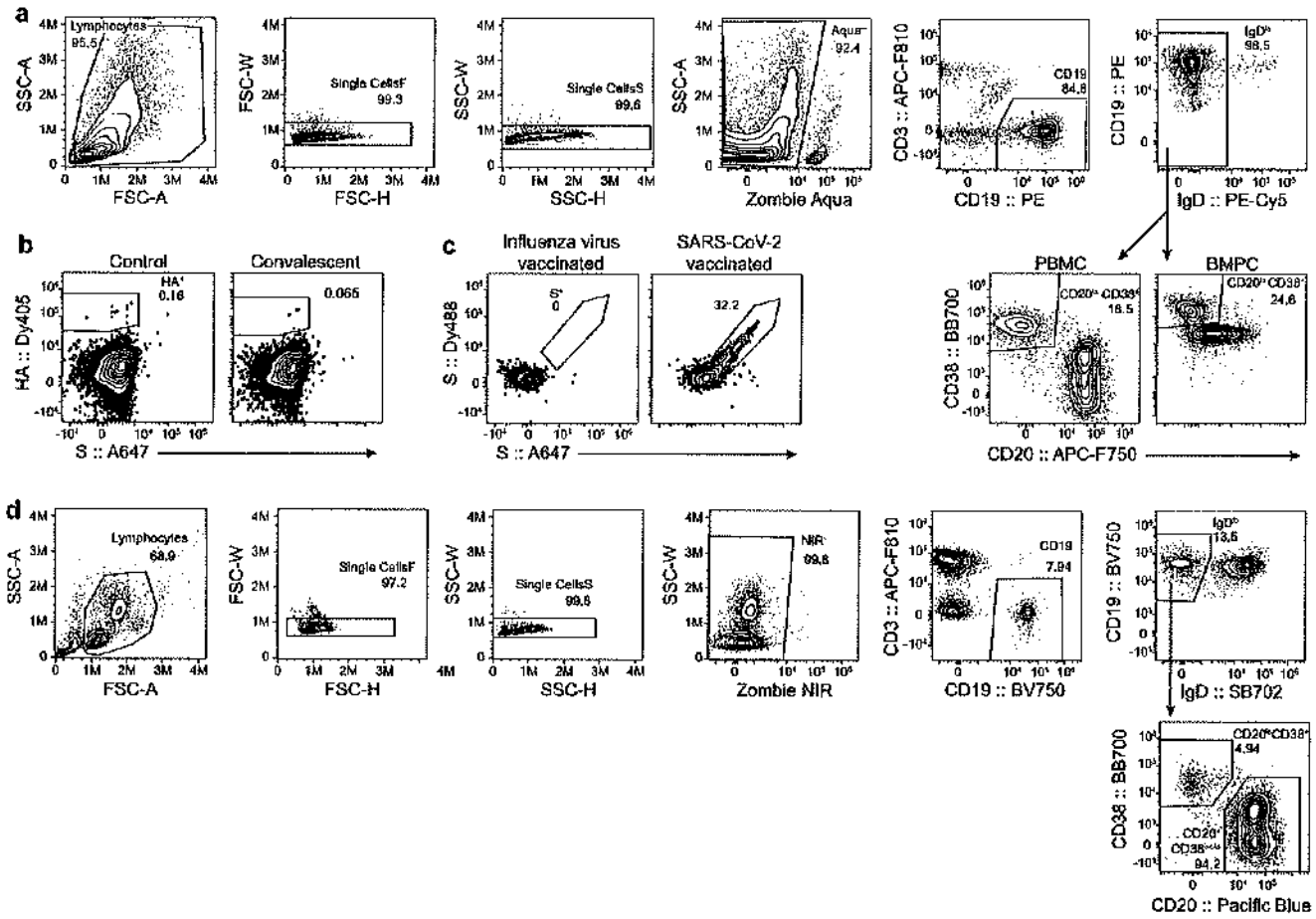

Extended Data Fig. 1 | Flow cytometry identification of SARS-CoV-2-elicited plasma cells and memory B cells. a, d, Flow cytometry gating strategies for BMPCs in magnetically enriched BMPCs and plasmablasts in PBMCs (**a**) and isotype-switched memory B cells and plasmablasts in PBMCs (**d**). **b,** Representative plots of intracellular SARS-CoV-2 S and influenza virus HA staining in BMPCs from samples from control individuals (left) and individuals who were convalescing from COVID-19 (right) 7 months after symptom onset. **c,** Representative plots of intracellular S staining in plasmablasts in PBMCs one week after vaccination against seasonal influenza virus or SARS-CoV-2.

Extended Data Table 1 | Demographics of patients with COVID-19

	Total N=77 N (%)	Bone marrow biopsy N=19 N (%)
Age (median [range])	49 (21-69)	52 (30-69)
Sex		
Female	38 (49.4)	7 (36.8)
Male	39 (50.6)	12 (63.2)
Race		
White	70 (90.9)	18 (94.7)
Black	1 (1.3)	0 (0)
Asian	4 (5.2)	0 (0)
Other	2 (2.6)	1 (5.3)
Comorbidities		
Asthma	13 (16.9)	3 (15.8)
Lung disease	0 (0)	0 (0)
Heart disease	3 (3.9)	0 (0)
Hypertension	13 (16.9)	6 (31.6)
Diabetes mellitus	3 (3.9)	3 (15.8)
Cancer	10 (13)	3 (15.8)
Autoimmune disease	4 (5.2)	2 (10.5)
Hyperlipidemia	8 (10.4)	2 (10.5)
Hypothyroidism	5 (6.5)	3 (15.8)
Gastroesophageal reflux disease	5 (6.5)	2 (10.5)
Other	26 (33.8)	10 (52.6)
Solid organ transplant	1 (1.3)	1 (5.3)
Obesity	1 (1.3)	0 (0)

Extended Data Table 2 | Symptoms of patients with COVID-19

	Total N=77 N (%)	Bone marrow biopsy N=19 N (%)
First symptom		
Cough	12 (15.6)	3 (15.8)
Diarrhea	1 (1.3)	0 (0)
Dyspnea	2 (2.6)	1 (5.3)
Fatigue	7 (9.1)	0 (0)
Fever	22 (28.6)	9 (47.4)
Headache	8 (10.4)	2 (10.5)
Loss of taste	3 (3.9)	2 (10.5)
Malaise	4 (5.2)	1 (5.3)
Myalgias	9 (11.7)	0 (0)
Nasal congestion	2 (2.6)	0 (0)
Nausea	1 (1.3)	0 (0)
Night sweats	1 (1.3)	0 (0)
Sore throat	5 (6.5)	1 (5.3)
Symptom present during disease		
Fever	65 (84.4)	17 (89.5)
Cough	54 (70.1)	14 (73.7)
Dyspnea	31 (40.3)	11 (57.9)
Nausea	19 (24.7)	4 (21.1)
Vomiting	9 (11.7)	3 (15.8)
Diarrhea	39 (50.6)	10 (52.6)
Headaches	47 (61)	12 (63.2)
Loss of taste	42 (54.5)	11 (57.9)
Loss of smell	42 (54.5)	10 (52.6)
Fatigue	38 (49.4)	7 (36.8)
Malaise	6 (7.8)	1 (5.3)
Myalgias or body aches	34 (44.2)	8 (42.1)
Sore throat	12 (15.6)	1 (5.3)
Chills	25 (32.5)	6 (31.6)
Nasal congestion	6 (7.8)	0 (0)
Other	32 (41.6)	7 (36.8)
Duration of symptoms in days (median [range])	14 (1-43)	13 (6-30)
Days from symptom onset to positive SARS-CoV-2 PCR test (median [range])	6 (0-36)	6 (1-31)
Days from symptom onset to 1-month blood sample collection (median [range])	41 (21-84)	34 (22-71)
Hospitalization	6 (7.8)	1 (5.3)
COVID medications		
Hydroxychloroquine	2 (2.6)	0 (0)
Chloroquine	1 (1.3)	0 (0)
Azithromycin	14 (18.2)	6 (31.6)
Lopinavir/ritonavir	0 (0)	0 (0)
Remdesivir	0 (0)	0 (0)
Convalescent plasma	0 (0)	0 (0)
None	61 (79.2)	12 (63.2)
Other	2 (2.6)	1 (5.3)

Extended Data Table 3 | Symptoms and follow up samples (months 4–11) of convalescent individuals

	Month 4		Month 7		Month 11	
	Total N= 76 N (%)	Bone marrow biopsy N=19 N (%)	Total N= 76 N (%)	Bone marrow biopsy N=18 N (%)	Total N= 42 N (%)	Bone marrow biopsy N=12 N (%)
Days from positive SARS-CoV-2 PCR test to follow up visit (median [range])	125 (102-192)	117 (105-150)	222 (191-275)	213 (200-247)	308 (283-369)	303 (283-325)
Days from symptom onset to blood sample collection (median [range])	131 (106-193)	124 (108-155)	227 (194-277)	222 (205-253)	314 (288-373)	309 (297-343)
Any symptom present at follow up visit	25 (32.9)	8 (42.1)	33 (43)	10 (55.6)	20 (47.6)	6 (50)
Fever	0 (0)	0 (0)	2 (2.6)	0 (0)	1 (2.4)	0 (0)
Cough	1 (1.3)	1 (5.3)	0 (0)	0 (0)	1 (2.4)	0 (0)
Dyspnea	7 (9.2)	2 (10.5)	6 (7.9)	3 (16.7)	6 (14.3)	3 (25)
Nausea	1 (1.3)	0 (0)	1 (1.3)	0 (0)	0 (0)	0 (0)
Vomiting	1 (1.3)	1 (5.3)	0 (0)	0 (0)	0 (0)	0 (0)
Diarrhea	2 (2.6)	1 (5.3)	1 (1.3)	0 (0)	0 (0)	0 (0)
Headaches	1 (1.3)	0 (0)	3 (3.9)	0 (0)	2 (4.8)	0 (0)
Loss or altered taste	8 (10.5)	0 (0)	9 (11.8)	1 (5.6)	5 (11.9)	1 (8.3)
Loss or altered smell	13 (17.1)	2 (10.5)	12 (15.8)	2 (11.1)	8 (19)	2 (16.7)
Fatigue	9 (11.8)	4 (21.1)	13 (17.1)	5 (27.8)	8 (19)	3 (25)
Forgetfulness/brain fog	8 (10.5)	6 (31.6)	12 (15.8)	6 (33.3)	10 (23.8)	4 (33.3)
Hair loss	5 (6.6)	1 (5.3)	3 (3.9)	1 (5.6)	2 (4.8)	0 (0)
Other	7 (9.2)	3 (15.8)	12 (15.8)	1 (5.6)	10 (23.8)	1 (8.3)
Joint pain	3 (3.9)	1 (5.3)	7 (9.2)	1 (5.3)	3 (7.1)	0 (0)

Extended Data Table 4 | Healthy control demographics

Variable	Total N= 11 N (%)
Age (median [range])	38 (23-53)
Sex	
Female	3 (27.3)
Male	8 (72.7)
Race	
White	9 (71.8)
Black	1 (9.1)
Asian	1 (9.1)

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Last updated by author(s): May 26, 2021

Reporting Summary

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Flow cytometry data were acquired using SpectroFlo software v2.2.
Data analysis	Flow cytometry data were analyzed using FlowJo v10 and Prism v8 ELISA and ELISpot data were analyzed using Prism v8 and SAS 9.4

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All relevant data are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to determine sample size. 77 convalescent patients and 11 control participants were enrolled based on recruitment; these numbers provided sufficient power to determine differences in SARS-CoV-2 responses between the groups.
Data exclusions	No data were excluded
Replication	Samples were collected from 77 convalescent patients and 11 control participants. ELISA for each participant at each timepoint was performed once with two technical replicates. ELISpot and flow cytometry experiments were performed once for each sample at each timepoint.
Randomization	Different experimental groups were not assigned.
Blinding	No blinding was done in this study; subjective measurements were not made.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	IgG-HRP (goat polyclonal, Jackson ImmuoResearch 109-035-088), IgG-BV480 (goat polyclonal, Jackson Immunoresearch 109-685-098), IgD-SB702 (IA6-2, Thermo 67-9868-42), IgA-FITC (M24A, Millipore CBL114F), CD45-A532 (HI30, Thermo 58-0459-42), CD38-BB700 (HIT2, BD Horizon 566445), Blimp1-A700 (646702, R&D IC36081N), CD20-Pacific Blue (2H7, 302320), CD4-BV570 (OKT4, 317445), CD24-BV605 (ML5, 311124), streptavidin-BV650 (405232), Ki-67-BV711 (Ki-67, 350516), CD19-BV750 (HIB19, 302262), CD19-PE (HIB19, 302254), CD71-PE (CY1G4, 334106), CXCR5-PE-Dazzle 594 (J252D4, 356928), CD27-PE-Cy7 (O323, 302838), CD71-PE-Cy7 (CY1G4, 334112), CD20-APC-Fire750 (2H7, 302358), IgM-APC-Fire750 (MHM-88, 314546), CD3-APC-Fire810 (SK7, 344858); all Biolegend.
Validation	Commercial antibodies were validated by their respective manufacturers per their associated data sheets and titrated in the lab for their respective assay (ELISA or flow cytometry) by serial dilution

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Expi293F (Thermo)
Authentication	The cell line was not authenticated
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination. Growth rates were consistent with manufacturer's published data.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used

Human research participants

Policy information about studies involving human research participants

Population characteristics	77 SARS-CoV-2 convalescent study participants were recruited, ages 21-69, 49.4% female, 50.6% male 11 healthy control participants with no history of SARS-CoV-2 infection were recruited, ages 23-53, 27.3% female, 72.7% male
Recruitment	Study participants were recruited from the St. Louis metropolitan area by the Washington University Clinical Trials Unit. Potential self-selection and recruiting biases are unlikely to affect the parameters we measured.
Ethics oversight	The study was approved by the Washington University IRB

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Peripheral blood and bone marrow mononuclear cells were isolated from EDTA anticoagulated blood and bone marrow aspirates, respectively using density gradient centrifugation, and remaining RBCs were lysed with ammonium chloride lysis buffer. Bone marrow plasma cells were magnetically enriched from bone marrow mononuclear cells and immediately used for ELISpot or cryopreserved in 10% dimethylsufoxide in FBS for flow cytometric analysis. PBMCs were immediately used or cryopreserved in 10% DMSO in FBS.
Instrument	Cytek Aurora
Software	Flow cytometry data were acquired using Cytek SpectroFlo software, and analyzed using FlowJo (Treestar) v10.
Cell population abundance	Cells were not sorted
Gating strategy	Gating strategies are shown in extended data figure

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Transmission potential of vaccinated and unvaccinated persons infected with the SARS-CoV-2 variant in a federal prison, July—August 2021

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Abstract

Background

The extent to which vaccinated persons who become infected with SARS-CoV-2 contribute to transmission is unclear. During a SARS-CoV-2 Delta variant outbreak among incarcerated persons with high vaccination rates in a federal prison, we assessed markers of viral shedding in vaccinated and unvaccinated persons.

Methods

Consenting incarcerated persons with confirmed SARS-CoV-2 infection provided mid-turbinate nasal specimens daily for 10 consecutive days and reported symptom data via questionnaire. Real-time reverse transcription-polymerase chain reaction (RT-PCR), viral whole genome sequencing, and viral culture was performed on these nasal specimens. Duration of RT-PCR positivity and viral culture positivity was assessed using survival analysis.

Results

A total of 978 specimens were provided by 95 participants, of whom 78 (82%) were fully vaccinated and 17 (18%) were not fully vaccinated. No significant differences were detected in duration of RT-PCR positivity among fully vaccinated participants (median: 13 days) versus those not fully vaccinated (median: 13 days; p=0.50), or in duration of culture positivity (medians: 5 days and 5 days; p=0.29). Among fully vaccinated participants, overall duration of culture positivity was shorter among Moderna vaccine recipients versus Pfizer (p=0.048) or Janssen (p=0.003) vaccine recipients.

Conclusions

As this field continues to develop, clinicians and public health practitioners should consider vaccinated persons who become infected with SARS-CoV-2 to be no less infectious than unvaccinated persons. These findings are critically important, especially in congregate settings where viral transmission can lead to large outbreaks.

Introduction

COVID-19 vaccines are highly effective in preventing severe illness and death from SARS-CoV-2 (the virus that causes COVID-19). However, because COVID-19 vaccines are not 100% effective in preventing infection, some infections among vaccinated persons are expected to occur. As global vaccination coverage increases, the role of vaccinated persons in transmission will be a critical determinant of the pandemic's future trajectory.[1] The extent to which vaccinated persons who become infected contribute to transmission of SARS-CoV-2, including the B.1.617.2 (Delta) variant, is not yet well understood. Some preprint manuscripts have reported comparable indicators of transmission potential regardless of vaccination status,[2] while others have reported reduced viability of virus isolated from vaccinated persons.[3]

The Delta variant has been associated with a peak in COVID-19 cases in the United States beginning in July 2021 that included large outbreaks among vaccinated and unvaccinated persons in crowded settings.[4-6] These findings are of particular concern for congregate living environments such as correctional and detention facilities and long-term care facilities because of the potential for rapid transmission of SARS-CoV-2 and the high prevalence of underlying health conditions associated with severe COVID-19.[7-9]

In a recent outbreak involving the Delta variant in a federal prison in Texas, the cumulative incidence of infection in two affected housing units was 74%; it was 93% and 70% among unvaccinated and vaccinated incarcerated persons, respectively.[6] Using serial mid-turbinate nasal specimens collected from a subset of incarcerated persons infected during this outbreak, this report assesses reverse transcription-polymerase chain reaction (RT-PCR) and viral culture characteristics as surrogate markers of transmission potential among persons fully vaccinated and those not fully vaccinated over time. This report is one of the first longitudinal investigations of viral shedding from vaccinated persons infected

with the Delta variant and contributes to the evidence base guiding infection prevention and control procedures across a variety of settings.

Methods

Investigational Setting

On July 12, 2021, an outbreak of SARS-CoV-2 among vaccinated and unvaccinated persons was detected in a federal prison in Texas. Staff from the Centers for Disease Control and Prevention (CDC) and Federal Bureau of Prisons (BOP) deployed to the prison to investigate the outbreak as previously reported.[6] As part of this outbreak investigation, a subset of incarcerated persons provided serial mid turbinate nasal specimens which were analyzed to evaluate the potential role of infected vaccinated and unvaccinated persons in transmission of SARS-CoV-2. This activity was reviewed and approved by the BOP Research Review Board and CDC and conducted consistent with applicable federal law and CDC policy.*

Participant Enrollment and Serial Specimen Collection

Incarcerated persons living in four housing units where COVID-19 cases had been identified were invited to participate in serial swabbing. Persons were eligible to enroll if they had tested positive for SARS-CoV-2 between July 12 (the start of the outbreak) and August 4, 2021. CDC and BOP staff held information sessions to explain the purpose of the project and to answer questions, including privacy protections and how results of the study would be made available to participants. All persons choosing to participate signed informed consent forms, which were provided in English and Spanish.

Specimen collection occurred during July 18—August 9, 2021. CDC and BOP staff collected one nasal mid-turbinate specimen daily for 10 consecutive days from participants who had tested positive,

beginning on July 19 or, for cases identified after July 19, beginning on the date of participants' first positive test. All incarcerated persons residing in housing units where cases were identified were placed under quarantine precautions. To assist in case-finding, consenting persons who were quarantined were tested every other day beginning on July 19 or on their first full day of quarantine; those who tested positive during quarantine were invited to participate in the 10 consecutive days of specimen collection. All participants were asked to provide a specimen on August 6 to provide data additional data on viral shedding, which corresponds to a late timepoint in infection for most participants (Figure 1).

On the tenth day of specimen collection, participants were asked to complete a paper-based questionnaire to report COVID-19-like symptoms during the course of their illness, including date of symptom onset and symptom duration. Information on demographic characteristics, COVID-19 vaccination history, previous positive SARS-CoV-2 diagnostic tests, and underlying medical conditions was extracted from BOP electronic medical records for all participants.

Laboratory Methods

Specimens were collected using nylon flocked minitip swabs, transferred into universal viral transport media (VTM) (Becton Dickinson, Franklin Lakes, NJ) immediately stored at 2-8°C and frozen at -20°C or colder within 72 hours, and sent to CDC for RT-PCR testing using the CDC Influenza SARS-CoV-2 Multiplex Assay. Remnant aliquots were stored at -70°C or below for viral culture. Due to capacity limitations, viral culture was performed on a subset of collected specimens. Specimens were included for viral culture if they had been collected 0, 3, 5, 7, or 9 days since onset and had an accompanying positive RT-PCR test with cycle threshold (Ct) value less than 35. For verification that this selected Ct cutoff did not exclude specimens containing culturable virus, viral culture was also performed on 25 of 102 specimens with Ct>35. (25/25 of these specimens were culture negative.) For more granular detail across the time-course of infection, viral culture was also performed on a subset of

specimens collected on other days (see Supplemental Figures 1-2 for details on specimens included for viral culture).

Specimens selected for culture were used to perform limiting-diluting inoculation of Vero CCL-81 cells expressing TMPRSS2, and cultures showing evidence of cytopathic effect were tested by RT-PCR for the presence of SARS-CoV-2 RNA. Viral recovery was as previously described.[10] Whole genome sequencing (WGS) was performed for one RT-PCR-positive specimen per participant with Ct less than 30 (per sequencing laboratory standard protocols).

Statistical Methods

Onset (used as time 0 in longitudinal analyses below) was defined to be either a) date of first onset of self-reported symptom(s) meeting the case definition of COVID-19,[11] or b) date of first positive diagnostic SARS-CoV-2 test, whichever occurred first. In two instances where a participant without symptoms had an initial positive test followed by at least 3 negative tests before subsequent positive tests, the date of second positive test was used.

Participants were considered fully vaccinated if ≥14 days had elapsed since they had completed all recommended doses of a COVID-19 primary vaccine series before the start of the outbreak. (No participant had completed a primary vaccine series <14 days before the outbreak.) Participants were considered not fully vaccinated if they had not received any doses of a vaccine or if they had not completed all doses of a vaccine series. Demographic characteristics of participants stratified by vaccination status were assessed using Fisher's exact tests.

Three surrogate markers for assessing transmission potential were analyzed as primary outcomes: RT-PCR positivity (an indicator of current/recent infection), RT-PCR Ct value (a semi-quantitative indicator of relative level of viral nucleic acid), and viral culture positivity (an indicator of viable/infectious virus). Dichotomous laboratory results (RT-PCR positivity and viral culture positivity)

were analyzed longitudinally with time 0 defined as the date of onset and the primary endpoints defined by a participant's last positive test. Specimens for which viral culture was not performed were presumed to be culture negative if an accompanying RT-PCR test was negative or was positive with Ct>35. To account for variation in the interval between onset and enrollment, and intermittent participation in specimen collection by some participants (which can result in interval and right censoring), survival analyses were performed using Turnbull estimation using the "interval" package implementation in R.[12] Hypothesis testing of survival functions was performed using the generalized Wilcoxon-Mann-Whitney method for interval-censored data.

As a post-hoc evaluation of potential interactions between vaccination status and known prior SARS-CoV-2 infections, a stratified analysis was conducted using Fisher's exact test to compare RT-PCR and viral culture results across these two variables among specimens collected on days with complete viral culture coverage (0, 3, 5, 7, and 9 days since onset).

Non-dichotomous laboratory results (RT-PCR Ct values) were characterized by days since onset using medians and interquartile ranges (IQRs). Because Ct values are semi-parametric, distributions were compared non-parametrically using the Mann-Whitney U test with ties (for dichotomous variables) or the Kruskal-Wallis test (for categorical variables with more than 2 levels); negative RT-PCR results were assigned higher ranks than any Ct value from positive RT-PCR results. To account for multiple hypothesis testing across days, α thresholds were adjusted using Bonferroni correction. All hypothesis tests performed are detailed in Supplementary Tables 1 and 2. All statistical analyses were performed in R version 4.0.2 (R Core Team, Vienna, Austria).

Results

Population Characteristics

Among 189 persons with SARS-CoV-2 infection eligible to enroll, a total of 96 persons consented to participate in serial specimen collection; one participant had a single positive diagnostic test (Ct=36.2) followed by seven negative diagnostic tests and reported no symptoms and was excluded as a non-case. Of the 95 included participants, 78 (82%) were documented as being fully vaccinated against SARS-CoV-2, 15 (16%) were unvaccinated and 2 (2%) were partially vaccinated and categorized as not fully vaccinated in further analyses (Table 1). Among fully vaccinated participants, a majority (57/78, 73%) received the Pfizer vaccine; smaller proportions received the Moderna vaccine (14/78, 18%) or Janssen vaccine (7/78, 9%). A majority (47/78, 60%) of fully vaccinated participants completed their vaccination series more than 120 days prior to the start of the outbreak (IQR: 81-140 days prior to start). Recipients of Pfizer vaccines completed their series earlier (IQR: 131-131 days) than recipients of Moderna (IQR: 81-82 days prior to start) or Janssen (IQR: 46-70 days prior to start) vaccines ($p<0.001$). A small number of participants (2/78 fully vaccinated, 3%, and 2/17 not fully vaccinated, 12%, $p=0.10$) had a documented prior SARS-CoV-2 infection. Based on symptom self-report at the end of sampling, 76% of participants reported at least one symptom in the COVID-19 case definition [CSTE 2021]. The most commonly reported symptoms were runny or stuffy nose (58%), loss of smell or taste (54%), and cough (45%). Of 95 specimens from 95 participants for which sequencing was attempted, 64 were successfully sequenced and passed quality metrics; all 64 (100%) belonged to the B.1.617.2 (Delta) lineage and AY.3 sublineage.

RT-PCR Positivity

From the 95 included participants, 978 specimens were collected for RT-PCR testing (825/978, 84% from fully vaccinated participants). Specimens were collected ranging from 13 days prior to onset (among participants tested during quarantine prior to diagnosis) to 32 days following onset. See Figure 1 for a diagrammatic representation of RT-PCR specimen collection from participants, and see

Supplemental Figure 1 for details of specimen collection by day since onset (stratified by vaccination status). A median of 6 days elapsed between onset and enrollment among fully vaccinated participants, compared with a median of 7 days among participants who were not fully vaccinated (p=0.33). Overall, 499 of the 978 (51%) specimens tested positive by RT-PCR.

No significant differences in time to last RT-PCR positive test were found. Median duration of RT-PCR positivity was 13 days among fully vaccinated participants versus 13 days among participants who were not fully vaccinated (p=0.50; Figure 2); and 10 days among participants with known history of prior SARS-CoV-2 infection (regardless of vaccination) versus 13 days among participants without any known prior infection (p=0.12). Among fully vaccinated participants, median duration of positivity was 10 days among Moderna vaccine recipients versus 13 days among Pfizer recipients and 13 days among Janssen recipients (p=0.39); and 13 days among participants fully vaccinated more than 120 days prior to the outbreak versus 11 days among participants vaccinated 120 days or less prior to the outbreak (p=0.32).

Ct Values

Ct values from specimens testing positive by RT-PCR increased with the number of days since onset (Figure 3). Among specimens from fully vaccinated participants, Ct values increased from a median of 26.4 (IQR: 23.5-28.4) on the day of onset to a median of 32.9 on day 10 (IQR: 30.5-34.6), while Ct values from specimens from participants who were not fully vaccinated increased from a median of 28.5 (IQR:24.8-31.8) on the day of onset to a median of 34.5 on day 10 (IQR: 29.4-35.2). Across the time-course of infection, no statistically significant difference was observed among Ct values by vaccination status on any day after Bonferroni correction (all p>0.0026, the Bonferroni-corrected α threshold). Additionally, no significant differences were observed among Ct values when stratified by vaccine product, time since vaccination, or known prior SARS-CoV-2 infection. While not statistically significant,

lower Ct values were observed early in the time-course of infection among Janssen vaccine recipients (day 3 median: 17.9; IQR: 17.6-19.4) than among Moderna (day 3 median: 27.4; IQR: 23.7-28.1) or Pfizer recipients (day 3 median: 24.8; IQR: 23.1-26.8; p=0.016 while Bonferroni α=0.0026).

Viral Culture Positivity

Of the 978 specimens collected, viral culture was performed on 286 (29%); an additional 556 (57%) were included as presumptive negative viral culture results due to an accompanying negative RT-PCR test (n=479) or a positive RT-PCR test with a Ct value greater than 35 (n=77). Viral culture capture by day since onset stratified by vaccination status is detailed in Supplementary Figure 2. Among the 842 specimens with a viral culture result, 75 (9%) had a positive viral culture. Virus was recovered from 57/690 (8%) of specimens from fully vaccinated participants, compared with 18/152 (12%) of specimens from participants who were not fully vaccinated (p=0.16).

No statistically significant difference was detected in the duration of viral culture positivity (Figure 4) between participants who were fully vaccinated (median: 5 days) compared with those who were not fully vaccinated (median: 5 days; p=0.29). (Viral culture results are illustrated as a function of days since onset and grouped by RT-PCR result in Supplementary Figure 4). Cumulative hazard functions indicate overall shorter culture positivity for fully vaccinated participants who received the Moderna vaccine than those who received Pfizer (p=0.048) or Janssen vaccines (p=0.003), but there was no significant difference between recipients of Pfizer and Janssen vaccines (p=0.12). No statistically significant differences in duration of culture positivity were detected when stratified according to time since vaccination (p=0.79) or known prior infection (p=0.99).

Factorial Stratification: Vaccination Status and History of Prior Infection

Figure 5 illustrates a post-hoc stratification of RT-PCR and viral culture results by vaccination status and prior SARS-CoV-2 infection. No statistically significant difference in RT-PCR or viral culture positivity was detected on any day; however, bivariate stratification resulted in small population sizes in some groups (n=2 participants each for those fully vaccinated with a known prior infection and those not fully vaccinated with a known prior infection), which limits the ability to draw conclusions about these groups.

Discussion

During a high-transmission outbreak of the SARS-CoV-2 Delta variant in a prison setting, we failed to find different durations of RT-PCR positivity, Ct values, or durations of viral culture positivity in fully vaccinated persons compared with persons who were not fully vaccinated. However, vaccinated persons who received the Moderna vaccine had a shorter duration of culture positivity compared with Pfizer or Janssen vaccine recipients. (However, Moderna vaccine recipients also were more recently vaccinated than Pfizer vaccine recipients.) Collectively, our findings suggest that, as evidence continues to emerge in this developing field, vaccinated persons who become infected should be regarded as not significantly less infectious than unvaccinated persons for the purposes of public health action.

As viral infections in vaccinated persons can result from either a failure to mount a protective immune response following initial vaccination or a gradual waning of immunological protection following initially robust protection, the infectiousness of vaccinated persons may be variable. It is plausible that some participants in this investigation who became infected despite vaccination had weak or waning vaccine-induced protection and were therefore similar to unvaccinated persons in the markers of transmission potential that we evaluated.

This report adds to a limited body of scientific literature evaluating the transmission potential of SARS-CoV-2 infections in vaccinated persons. Reports of infections in vaccinated persons have found mixed results using markers of transmission potential, and no longitudinal studies of viral culture characteristics in vaccinated persons with Delta infections have been published. A multi-site serial testing investigation involving Alpha (B.1.1.7) and Gamma (P.1) infections found that duration of culture positivity was shorter among vaccinated persons compared with unvaccinated persons.[13, 14] One report using surveillance data found lower Ct values among unvaccinated persons, but this difference was only observed for two of three RT-PCR probes and only during one of three months.[15] One cross-sectional report found no difference in Ct value by vaccination status.[2] However, extrapolating from cross-sectional and surveillance data may be challenging without data to account for timing of specimen collection in the course of infection. Nevertheless, this finding is corroborated by analysis of a clinical convenience sample which found vaccination did not impact Ct values and reduced viral recovery of Alpha variant but did not reduce recovery of Delta variant virus;[16] similar findings were mirrored by two retrospective health-system cohorts.[17, 18] A report of health system workers found that viral culture positivity was reduced in vaccinated persons despite similar Ct values as those in unvaccinated persons.[3] A separate report found that early in the clinical course of infection, Ct values were comparable between vaccinated and unvaccinated persons, but among individuals who presented to care later in their course of illness, Ct values were higher in vaccinated persons.[19] A study of household transmission of Delta infections found similar peak viral loads regardless of vaccination status, but noted faster declines in vaccinated persons.[20] Cumulatively, available data have not clearly or consistently identified markers of reduced transmission potential in vaccinated persons with SARS-CoV-2 infection. This report, which to our awareness represents the first longitudinal investigation of viral culture characteristics of vaccinated persons with Delta variant infections, further demonstrates the potential of vaccinated persons to contribute to SARS-CoV-2 transmission.

294 While our investigation did not find evidence of reduced transmission potential from vaccinated
295 persons with infection, vaccination is known to reduce the risk of infection,[6, 21] which prevents
296 secondary transmission. In addition, vaccination remains a strongly protective factor against morbidity
297 and mortality due to SARS-CoV-2.[22] Protection against infection, morbidity, and mortality underscores
298 the importance of maximizing vaccination coverage, particularly in settings where challenges to physical
299 distancing can result in rapid, widespread transmission when infections do occur.

300 The evidence that vaccinated persons can transmit SARS-CoV-2 to others suggests that there is
301 continued risk of widespread outbreaks when the virus is introduced into congregate settings, even
302 when vaccination coverage is high. In particular, because of the potential for rapid transmission and high
303 prevalence of underlying health conditions in incarcerated populations,[7, 8] persons living or working in
304 correctional facilities should quarantine after exposure to SARS-CoV-2, regardless of vaccination status.
305 Post-exposure quarantine is especially important where the risk of transmission is high (e.g., in dorm-
306 style housing, and where staff and/or incarcerated persons frequently interact across housing units) or
307 where the population is at high risk of severe outcomes from COVID-19. Facilities can continue to
308 minimize the need for quarantine by enforcing consistent indoor masking to the extent possible,
309 continuing recommended disinfection, cleaning, and ventilation, and maintaining routine test-based
310 screening programs that can identify cases early and facilitate timely action (including isolation) to limit
311 exposure to others. Facilities that implement routine test-based screening should continue to include
312 vaccinated persons in their frame.

313 This report is subject to several limitations. Due to the small proportion of participants who
314 were not fully vaccinated (19%), statistical comparisons on the basis of vaccination status were
315 underpowered, and negative findings reported here warrant cautious interpretation. To increase the
316 sample size of this group, two partially vaccinated participants were included, potentially diluting the
317 characteristics of unvaccinated participants. However, our conclusions did not change when analyses

were performed excluding these two participants. Similarly, only four participants had known prior infection, of which a higher proportion occurred in those not fully vaccinated; therefore, these participants may appear to have slightly greater immunological protection than those without prior infection. On average, unvaccinated participants enrolled earlier in the outbreak and later in their course of infection than vaccinated participants; we utilized Turnbull estimation in survival analyses to account for the possibility of interval censoring in this population. All symptom data was self-reported and collected at the end of the specimen collection period, which may have impacted the accuracy of participants' recall related to the date of symptom onset. Ct values are semi-quantitative indicators of viral RNA levels and cannot be interpreted as quantitative markers of viral load or infectiousness. To avoid drawing quantitative conclusions around Ct values, we conservatively utilized non-parametric rank-based statistics (Mann-Whitney and Kruskal-Wallis) with Bonferroni correction to describe Ct values in this investigation. Information on prior SARS-CoV-2 infection was obtained from medical records; persons with earlier infections that were undiagnosed or diagnosed prior to incarceration and not documented in the BOP medical system may not have been correctly characterized. Finally, we did not attempt viral culture for 561 specimens with Ct>35 and classified them as presumptively negative. This decision was based on negative viral culture results from 25/25 specimens with Ct>35 for which viral culture was performed during this investigation, as well as previously published findings demonstrating an inability to recover viable virus from specimens that were RT-PCR negative.[23]

In this investigation, we found no statistically significant difference in transmission potential between vaccinated persons and persons who were not fully vaccinated. Therefore, our findings indicate that prevention and mitigation measures should be applied without regard to vaccination status for persons in high-risk settings or those with significant exposures. In congregate settings, and correctional and detention facilities in particular, post-exposure testing and quarantine remain essential tools to limit transmission when cases are identified, in addition to other recommended prevention

342 measures.[24] Our data add to a growing body of evidence characterizing transmission potential from
343 vaccinated persons. Future studies of transmission potential from vaccinated persons with infection,
344 incorporating similar laboratory-based markers as well as evidence of transmission from secondary
345 attack rates and network analysis, may help to further describe the contributions of vaccinated persons
346 in chains of transmission as the pandemic evolves and new variants emerge.

Conflict of Interest Statement

The authors have no conflicts of interest to report. All authors have completed the ICMJE Conflict of Interest declaration.

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Footnotes

* 45 C.F.R. part 46, 21 C.F.R. part 56; 42 U.S.C. Sect. 241(d); 5 U.S.C. Sect. 552a; 44 U.S.C. Sect. 3501 et seq.

359 **Table 1. Characteristics of enrolled participants who tested positive for SARS-CoV-2, Federal prison,**
360 **Texas, July 12—August 9, 2021**

	All participants		Fully vaccinated		Not fully vaccinated*		p-value†
	n	%	n	%	n	%	
Total	95	100%	78	81%	17	19%	
Sex							
Male	95	100%	78	100%	17	100%	
Age							0.4
18-29	5	5%	3	4%	2	12%	
30-39	22	23%	19	24%	3	18%	
40-49	28	29%	22	28%	6	35%	
50-59	25	26%	20	26%	5	29%	
≥ 60	15	16%	14	18%	1	6%	
Race/Ethnicity							**0.008**
American Indian/Alaska Native	2	2%	2	3%	0	0%	
Asian	1	1%	1	1%	0	0%	
Black	16	17%	8	10%	8	47%	
Hispanic	12	13%	10	13%	2	12%	
White	64	67%	57	73%	7	41%	
Country of birth							0.6
Non US-born	4	4%	3	4%	1	6%	
US-born	91	96%	75	96%	16	94%	
Vaccination status							
Fully vaccinated	78	82%	78	100%	0	0%	
Not fully vaccinated*	17	18%	0	0%	17	100%	
Partially vaccinated	2	2%	0	0%	2	12%	
Unvaccinated	15	16%	0	0%	15	88%	
Vaccine product received							
Janssen	7	7%	7	9%	0	0%	
Moderna	14	15%	14	17%	0	0%	
Pfizer	57	60%	57	74%	0	0%	
Time from full vaccination to outbreak (if fully vaccinated)							
≤120 days	31	33%	31	33%	0	0%	
>120 days	47	49%	47	61%	0	0%	
Medical comorbidities							
Overweight‡	31	33%	24	31%	7	41%	
Obesity‡	47	49%	42	54%	5	29%	0.3
Severe obesity ‡	7	7%	6	8%	1	6%	
History of smoking	46	48%	42	54%	4	24%	**0.03**
Hypertension	43	45%	38	49%	5	29%	0.1

Diabetes	15	16%	14	18%	1	6%	0.3
Moderate/severe asthma	10	11%	8	10%	2	12%	1.0
Chronic obstructive pulmonary disease	6	6%	6	8%	0	0%	0.6
Cancer	1	1%	1	1%	0	0%	1.0
Chronic kidney disease	2	2%	2	3%	0	0%	1.0
Immunocompromised state	2	2%	2	3%	0	0%	1.0
HIV	0	0%	0	0%	0	0%	
Serious cardiac conditions	0	0%	0	0%	0	0%	
Liver disease	0	0%	0	0%	0	0%	
Documented prior SARS-CoV-2 infection							0.1
No	91	96%	76	97%	15	88%	
Yes	4	4%	2	3%	2	12%	
COVID-19 disease outcomes							
Hospitalization	2	2%	1	1%	1	6%	
Death	0	0%	0	0%	0	0%	
Reported Symptoms							
Reported any symptoms in CSTE case definition§	66	70%	54	70%	12	71%	0.7
Reported any symptoms	72	76%	59	76%	13	76%	0.4
Runny/Stuffy Nose	55	58%	48	62%	7	41%	0.4
Loss of Smell or Taste	51	54%	43	55%	8	44%	1.0
Cough	43	45%	35	45%	8	47%	0.8
Headache	40	42%	33	42%	7	41%	1.0
Muscle Aches	40	42%	30	38%	10	59%	0.08
Subjective Fever	34	36%	27	35%	7	41%	0.6
Measured Fever	10	11%	6	8%	4	24%	0.06
Chills	29	31%	21	27%	8	47%	0.06
Sore Throat	24	25%	21	27%	3	18%	0.7
Shortness of Breath	20	21%	14	18%	6	35%	0.08
Abdominal Pain, Nausea, Vomiting	17	18%	12	15%	5	28%	0.2
Diarrhea	16	17%	11	14%	5	28%	0.1
Other	6	6%	6	8%	0	0%	1.0
None Reported ¶	23	24%	19	24%	4	24%	1.0

*Not fully vaccinated participants include 15 who have not received any dose of a SARS-CoV-2 vaccine and 2 who receive only the first dose of a two-dose SARS-CoV-2 vaccine series.

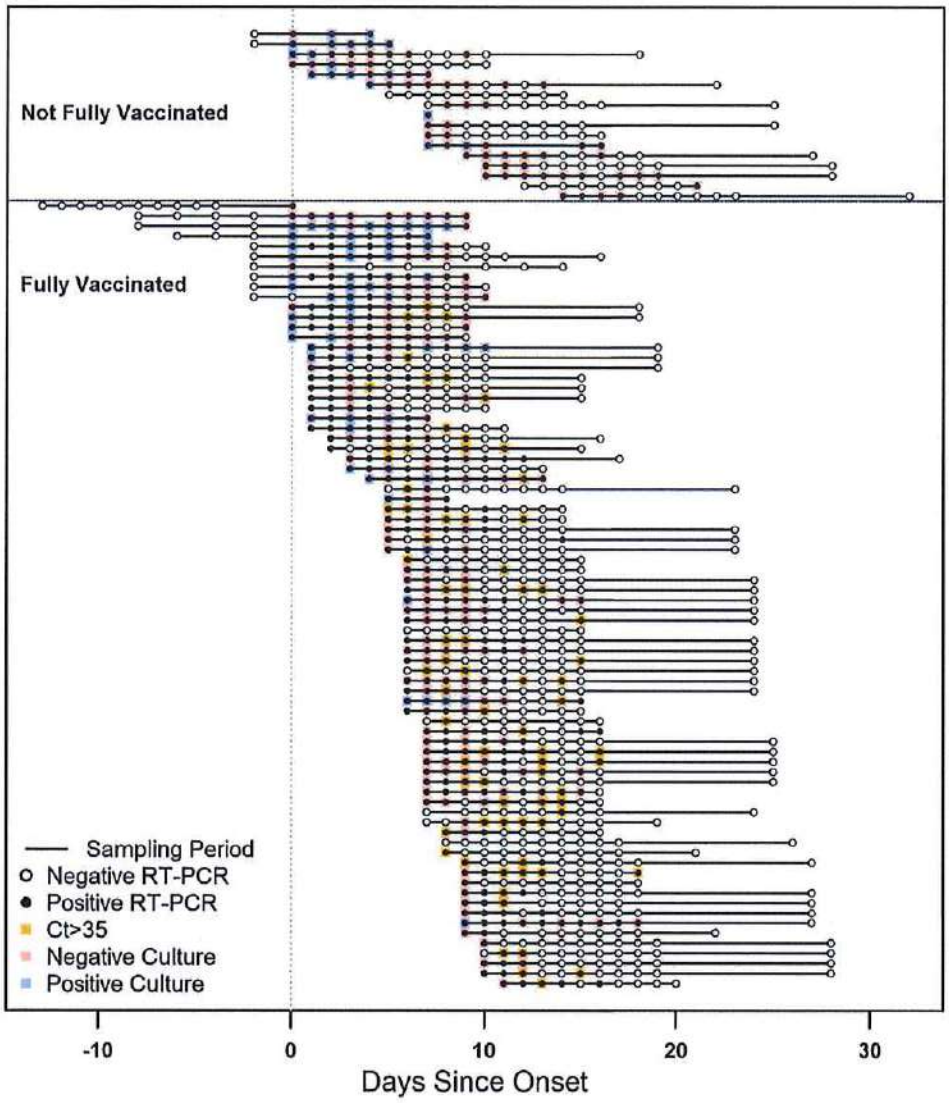
†P-values correspond to results of Fisher's exact tests.

‡Overweight was defined as a body mass index (BMI) >25 kg/m2 but <30 kg/m2; obesity was defined as BMI ≥30 kg/m2 but <40 kg/m2; severe obesity was defined as BMI ≥40 kg/m2.

§The COVID-19 case definition of the Council of State and Territorial Epidemiologists (CSTE) includes fever, chills, muscle aches, headache, sore throat, nausea/vomiting, diarrhea, fatigue, stuffy/runny nose, cough, shortness of breath, or loss of taste or smell. [CSTE 2021]

¶ 8 participants (5 fully vaccinated and 3 not fully vaccinated) declined to report symptoms in addition to 15 (14 and 1, respectively) who reported that they had no symptoms

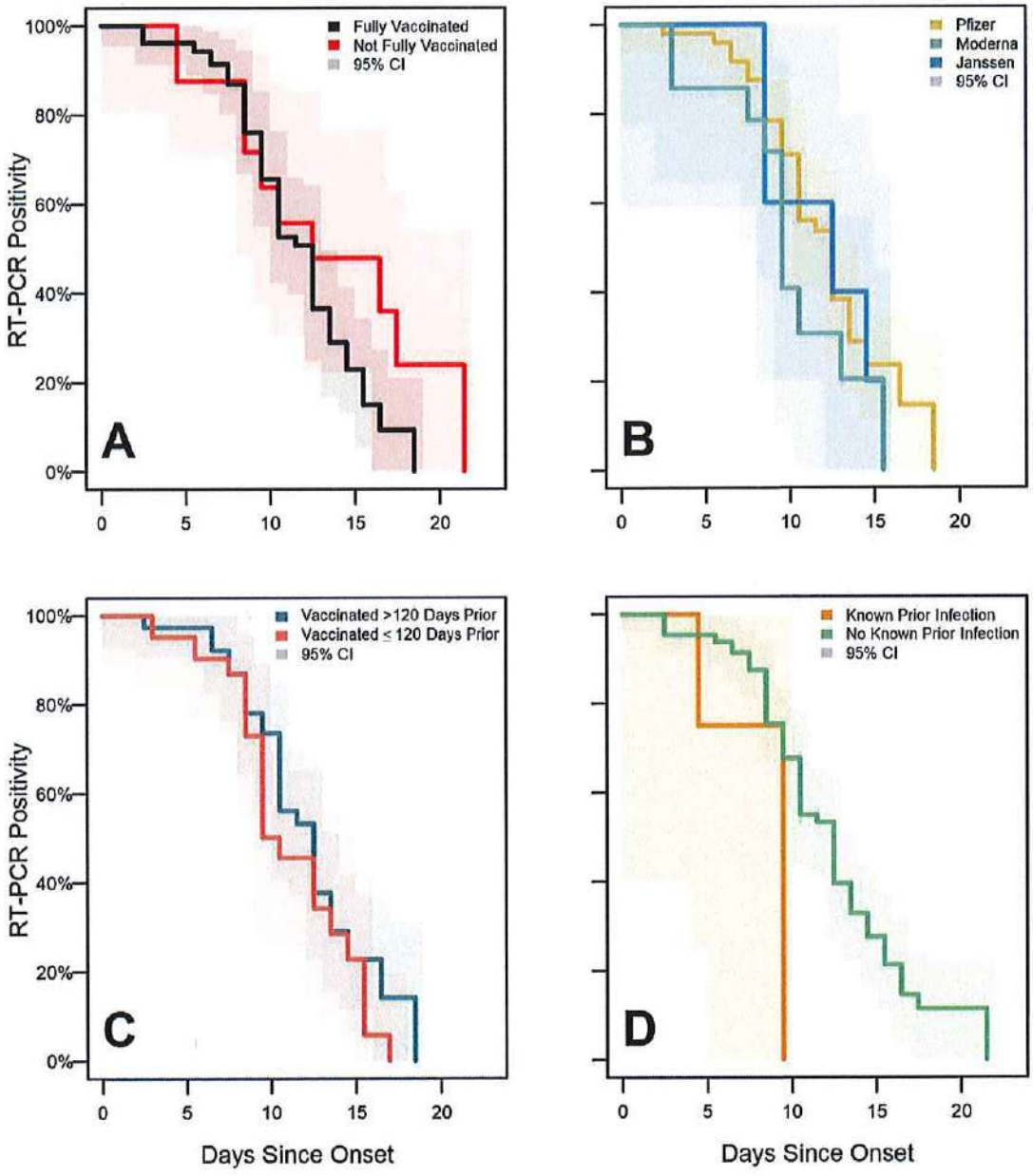
371 **Figure 1. Timelines and results of nasal mid-turbinate specimens collected from enrolled participants,**
372 **Federal prison, Texas, July 12—August 9, 2021**



373

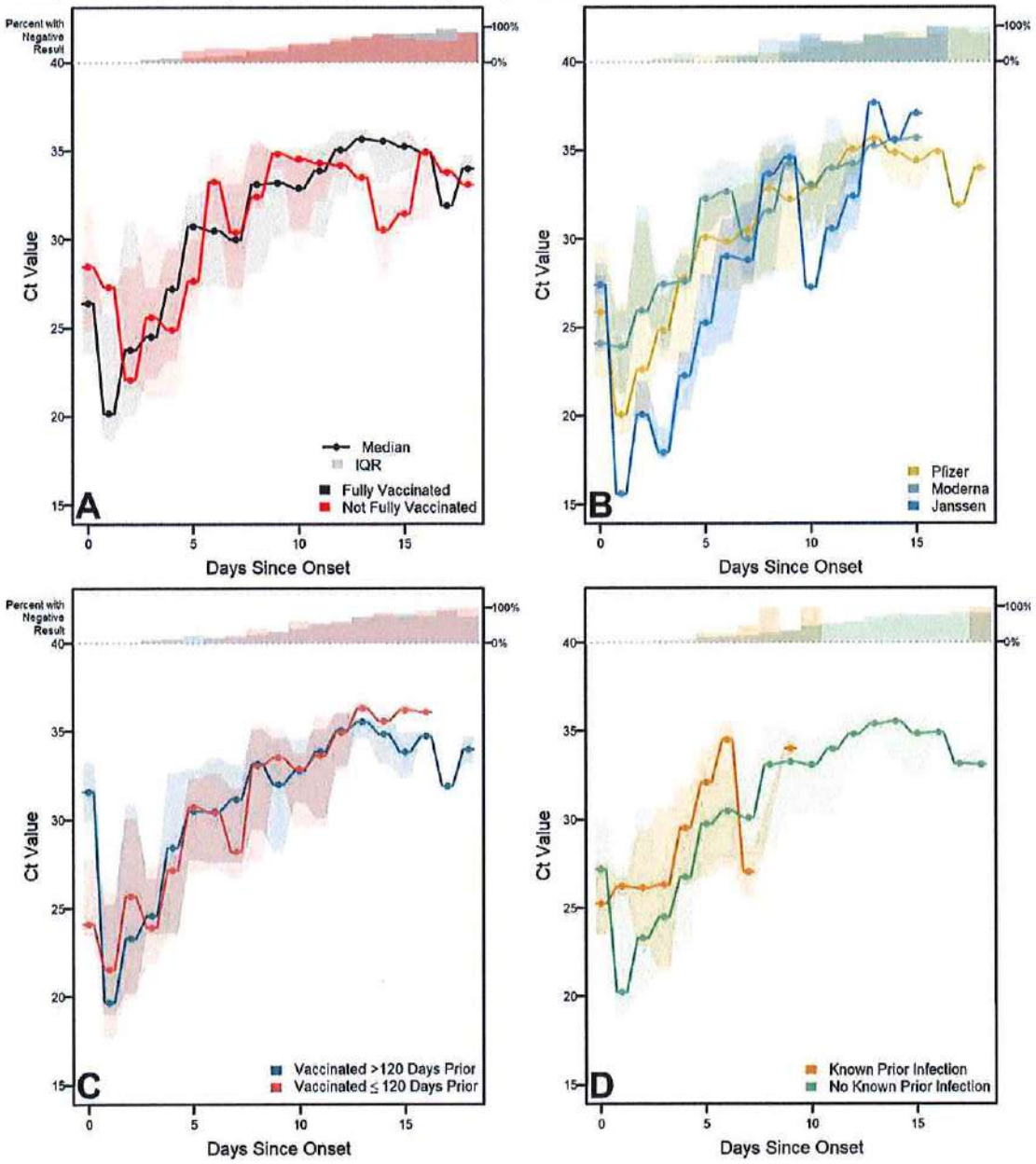
374 The timelines of specimen collection and laboratory results for 95 included participants are represented
375 diagrammatically, indexed by the day of onset. Onset was determined to be either a) date of first onset of self-
376 reported symptom(s) meeting the case definition of COVID-19 or b) date of first positive diagnostic SARS-CoV-2
377 test, whichever occurred first. Each participant is represented by a horizontal line corresponding to the
378 investigation sampling period during their time-course of illness. Participants who were not fully vaccinated
379 (including 2 participants who received only the first dose of a two-dose COVID-19 vaccine series) are depicted at
380 the top of the figure, while fully vaccinated participants are depicted at the bottom. RT-PCR results are
381 represented by solid circles (positive results) or open circles (negative results). For specimens with positive RT-PCR
382 results for which viral culture was performed, culture results are indicated by overlaid blue boxes (positive culture
383 results) or red boxes (negative culture results). Specimens with positive RT-PCR results with a cycle threshold (Ct)
384 value greater than 35 for which viral culture was not performed are indicated by overlaid orange boxes (indicated
385 a presumptive negative viral culture result). Some participants provided specimens during case-finding testing
386 while in quarantine and may have RT-PCR negative specimens collected prior to onset.

Figure 2. SARS-CoV-2 RT-PCR test positivity survival curves for enrolled participants, Federal prison, Texas, July 12—August 9, 2021



Panels illustrate the results of Turnbull estimation survival functions with a primary endpoint of last positive reverse transcription-polymerase chain reaction (RT-PCR) test result. Solid lines indicate nonparametric maximum likelihood estimates and shaded regions correspond to 95% confidence intervals estimated through modified bootstrap. Survival functions are plotted by Turnbull interval midpoints. Onset was determined to be either a) date of first onset of self-reported symptom(s) meeting the case definition of COVID-19 or b) date of first positive diagnostic SARS-CoV-2 test, whichever occurred first. Panel A depicts RT-PCR positivity by vaccination status (not fully vaccinated participants include 2 participants who received only the first dose of a two-dose COVID-19 vaccine series). Panel B depicts positivity by vaccine product among fully vaccinated participants. Panel C depicts positivity according to the time from completion of a COVID-19 vaccine/series to onset. Panel D depicts positivity according to history of known prior SARS-CoV-2 infection.

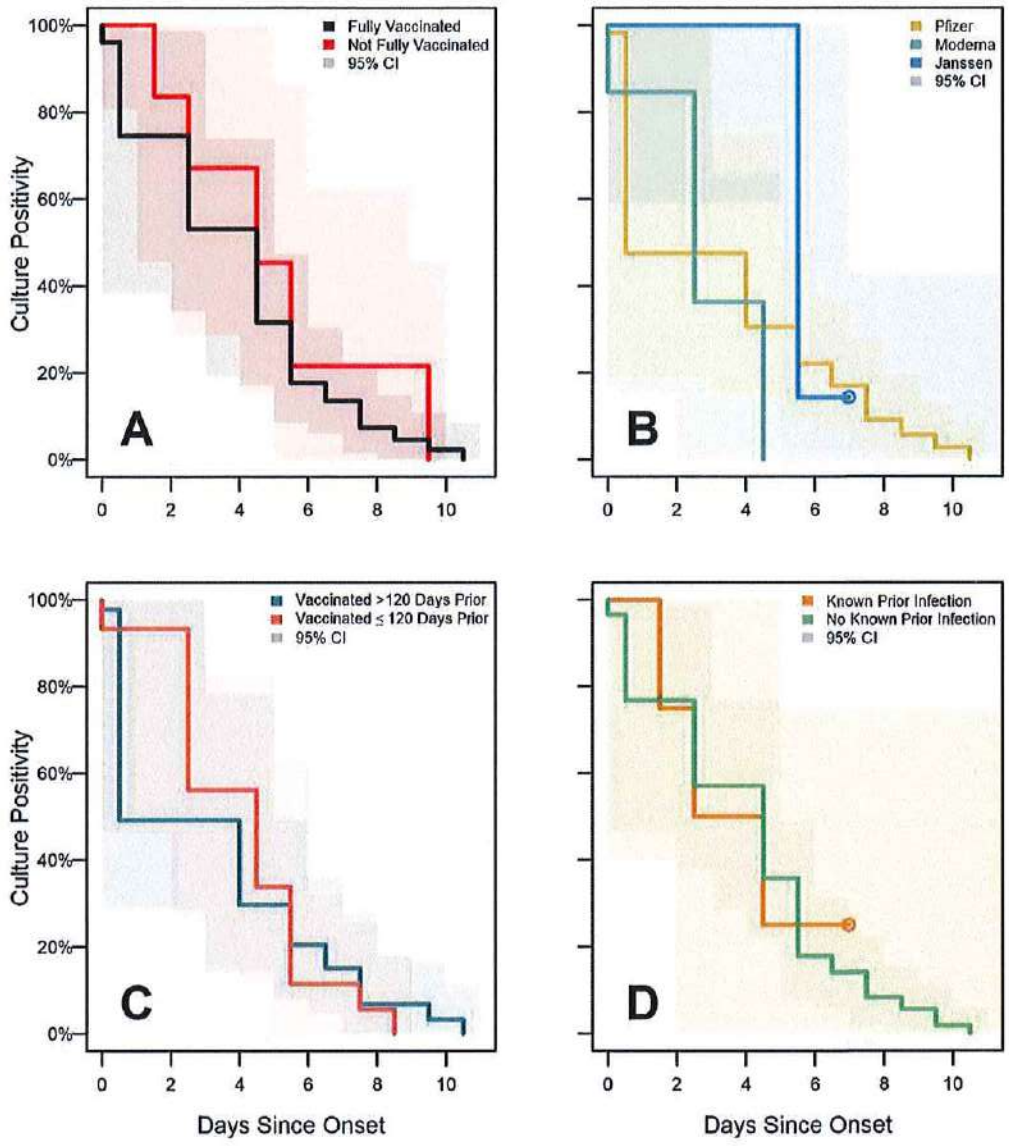
400 **Figure 3. RT-PCR Cycle Threshold distributions for enrolled participants with confirmed SARS-CoV-2**
401 **infection, Federal prison, Texas, July 12—August 9, 2021**



402

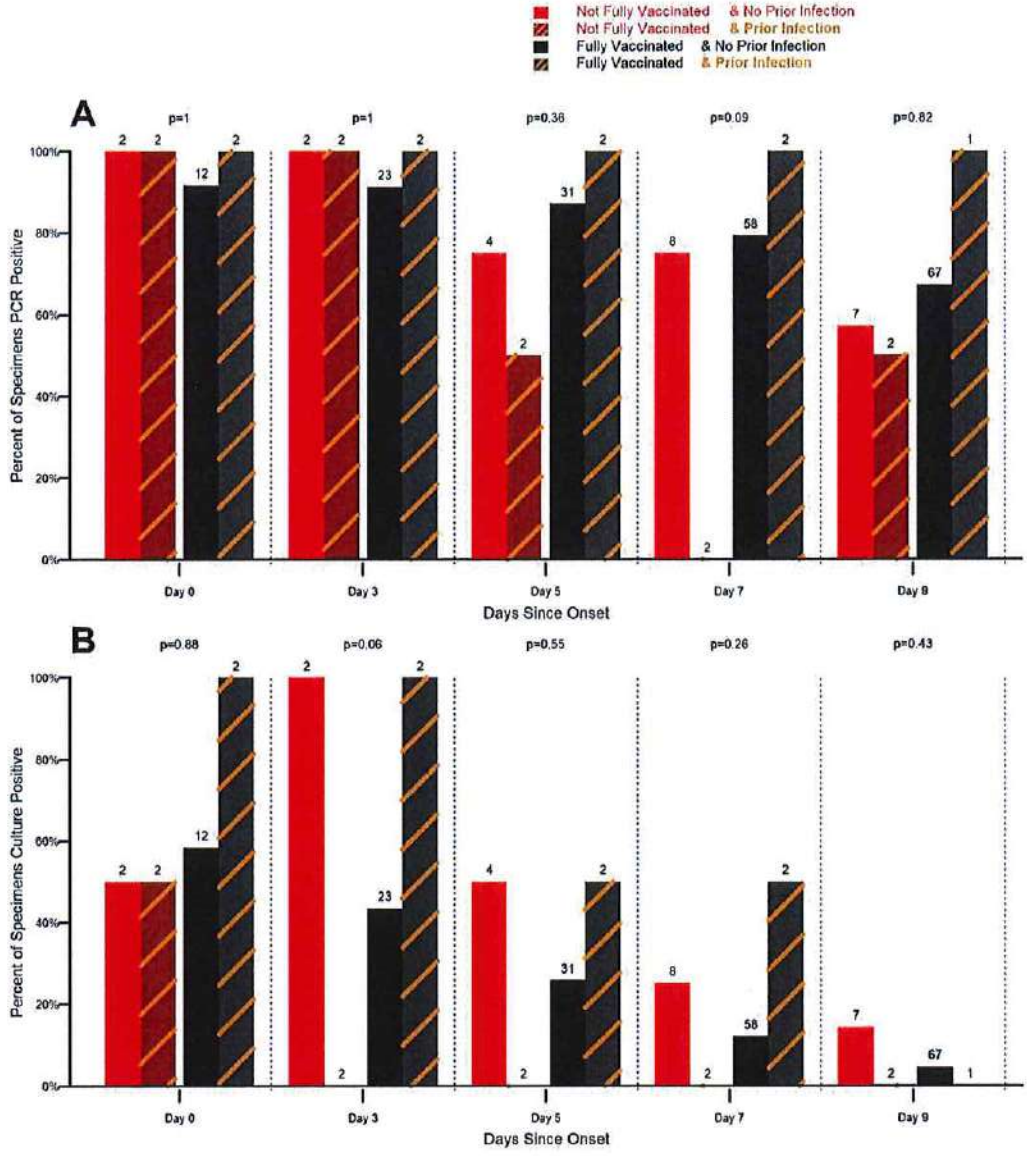
403 Panels illustrate daily medians and interquartile ranges (IQRs) for reverse transcription-polymerase chain reaction
404 (RT-PCR) cycle threshold (Ct) values among specimens with positive RT-PCR results. Solid lines indicate median Ct
405 values and shaded regions indicate IQRs. Percentages at the top of each panel indicate the proportion of
406 specimens with negative RT-PCR results each day Onset was determined to be either a) date of first onset of self-
407 reported symptom(s) meeting the case definition of COVID-19 or b) date of first positive diagnostic SARS-CoV-2
408 test, whichever occurred first. Panel A depicts RT-PCR positivity by vaccination status (not fully vaccinated
409 participants include 2 participants who received only the first dose of a two-dose COVID-19 vaccine series). Panel B
410 depicts positivity by vaccine product among fully vaccinated participants. Panel C depicts positivity according to
411 the time from completion of a COVID-19 vaccine/series to onset. Panel D depicts positivity according to history of
412 known prior SARS-CoV-2 infection.

Figure 4. SARS-CoV-2 viral culture test positivity survival curves for enrolled participants, Federal prison, Texas, July 12—August 9, 2021



Panels illustrate the results of Turnbull estimation survival functions with a primary endpoint of last positive viral culture test result. Specimens were included as presumptive negative results if no culture was performed but were accompanied by negative RT-PCR results or positive RT-PCR results with Ct>35. Solid lines indicate nonparametric maximum likelihood estimates and shaded regions correspond to 95% confidence intervals estimated through modified bootstrap. Survival functions are plotted by Turnbull interval midpoints. When Turnbull intervals are bounded by positive infinity (resulting from right-censoring in subgroups), survival functions are truncated by open points at the rightmost non-infinite intervals. Onset was determined to be either a) date of first onset of self-reported symptom(s) meeting the case definition of COVID-19 or b) date of first positive diagnostic SARS-CoV-2 test, whichever occurred first. Panel A depicts RT-PCR positivity by vaccination status (not fully vaccinated participants include 2 participants who received only the first dose of a two-dose COVID-19 vaccine series). Panel B depicts positivity by vaccine product among fully vaccinated participants. Panel C depicts positivity according to the time from completion of a COVID-19 vaccine/series to onset. Panel D depicts positivity according to history of known prior SARS-CoV-2 infection.

Figure 5. SARS-CoV-2 RT-PCR test positivity (A) and viral culture test positivity (B) stratified by vaccination status and prior infection status for enrolled participants, Federal prison, Texas, July 12—August 9, 2021



Panels illustrate the proportions of specimens for which RT-PCR test results (panel A) or viral culture test results (panel B) were positive, stratified by both vaccination status and history of prior SARS-CoV-2 infection. Solid bars indicate results for participants with no known prior infections, and striped bars indicate results for participants with documented prior infections. Specimens were included as presumptive negative results if no culture was performed but were accompanied by negative RT-PCR results or positive RT-PCR results with Ct>35. Onset was determined to be either a) date of first onset of self-reported symptom(s) meeting the case definition of COVID-19 or b) date of first positive diagnostic SARS-CoV-2 test, whichever occurred first. Results are depicted only for days 0, 3, 5, 7, and 9 since onset, representing days for which 100% of eligible specimens had viral culture performed. Bar labels indicate the number of specimens collected from participants in each group for each day. P-values are reported at the top of each daily grouping and correspond to Fisher's exact test of proportions across the four groups.

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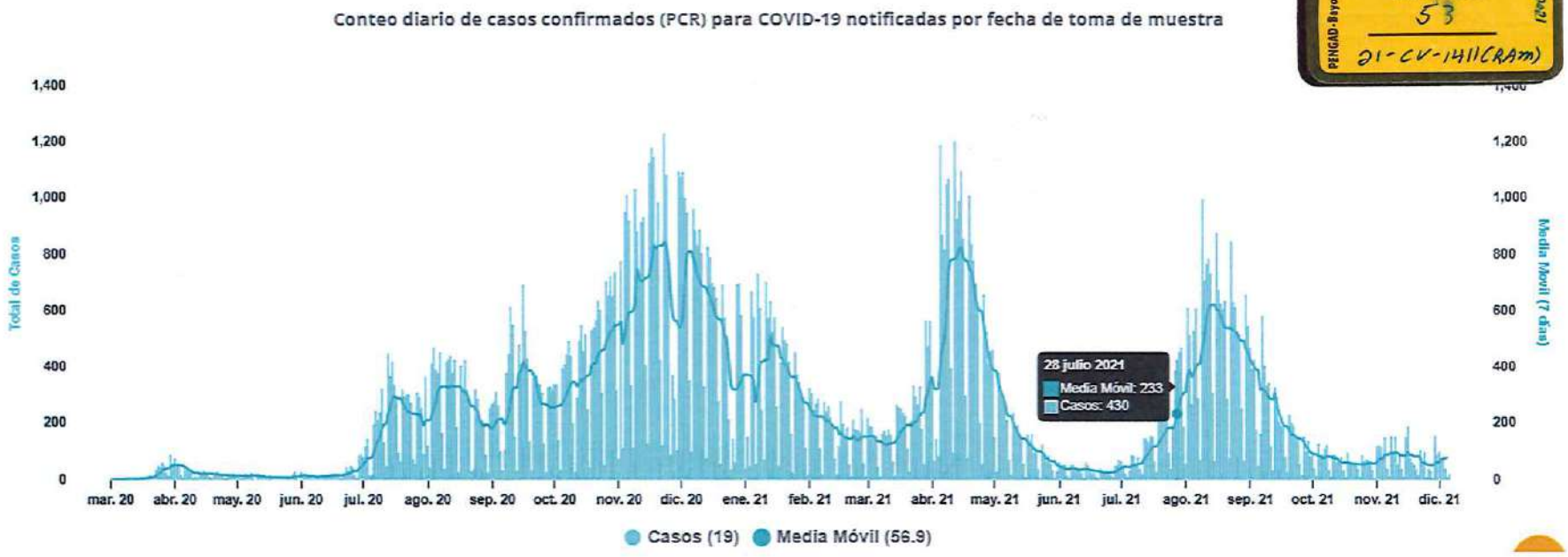
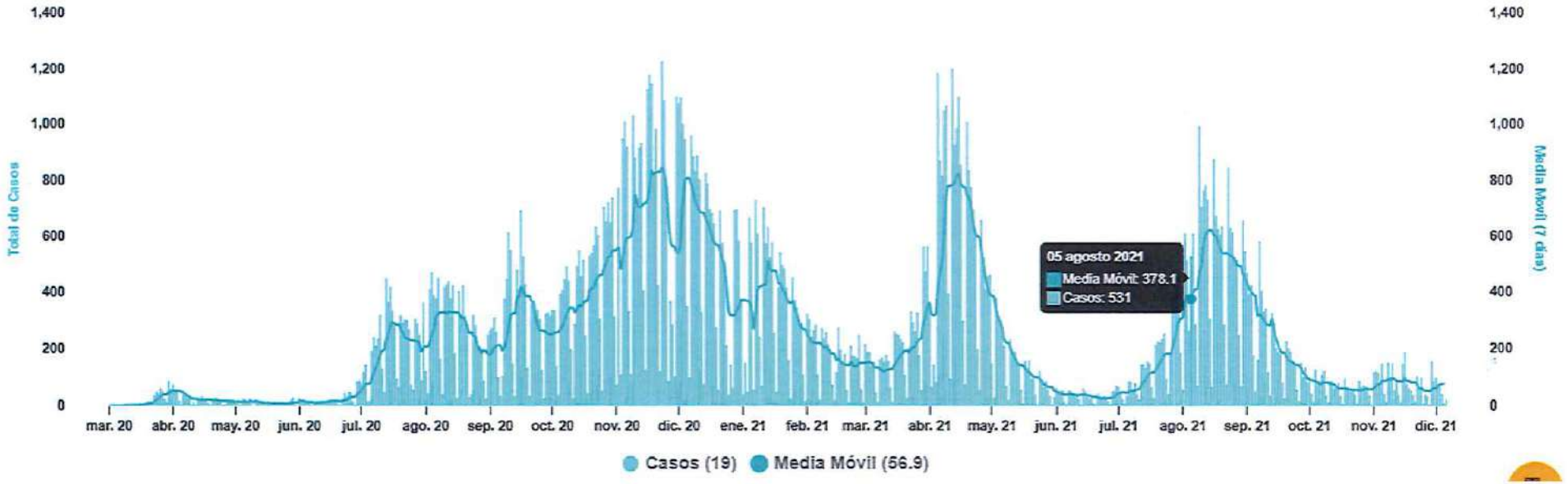
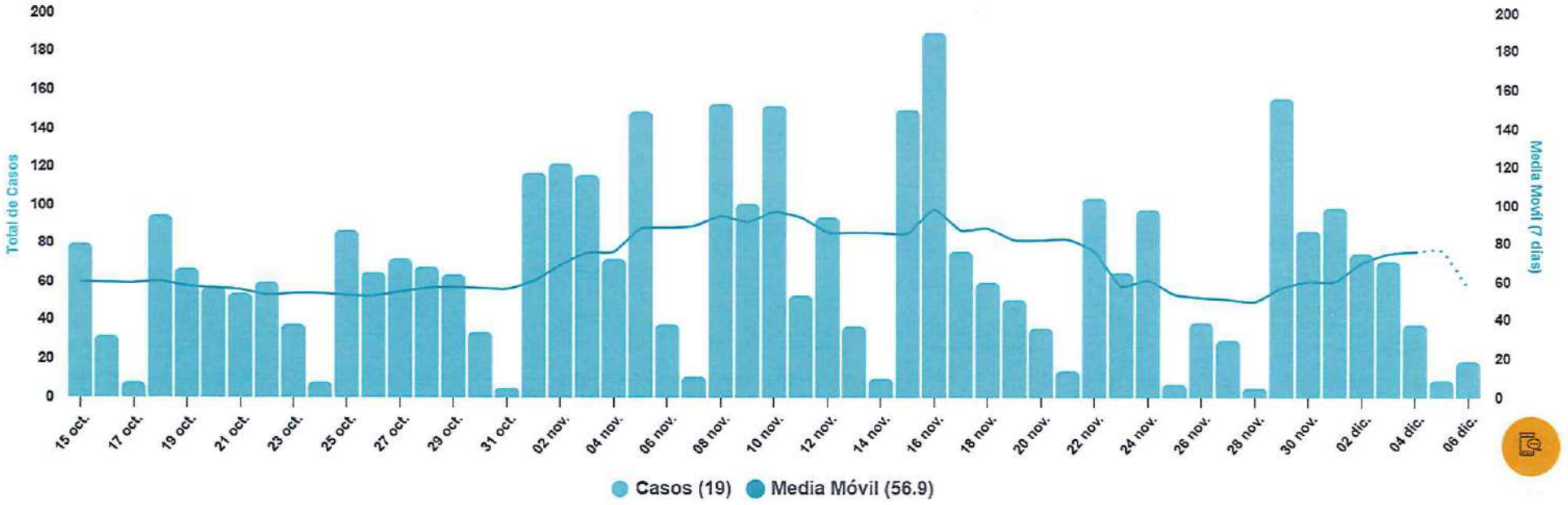




Puerto Rico Health Department, COVID-19 Dashboard, *Casos*, https://covid19datos.salud.gov.pr/#casos

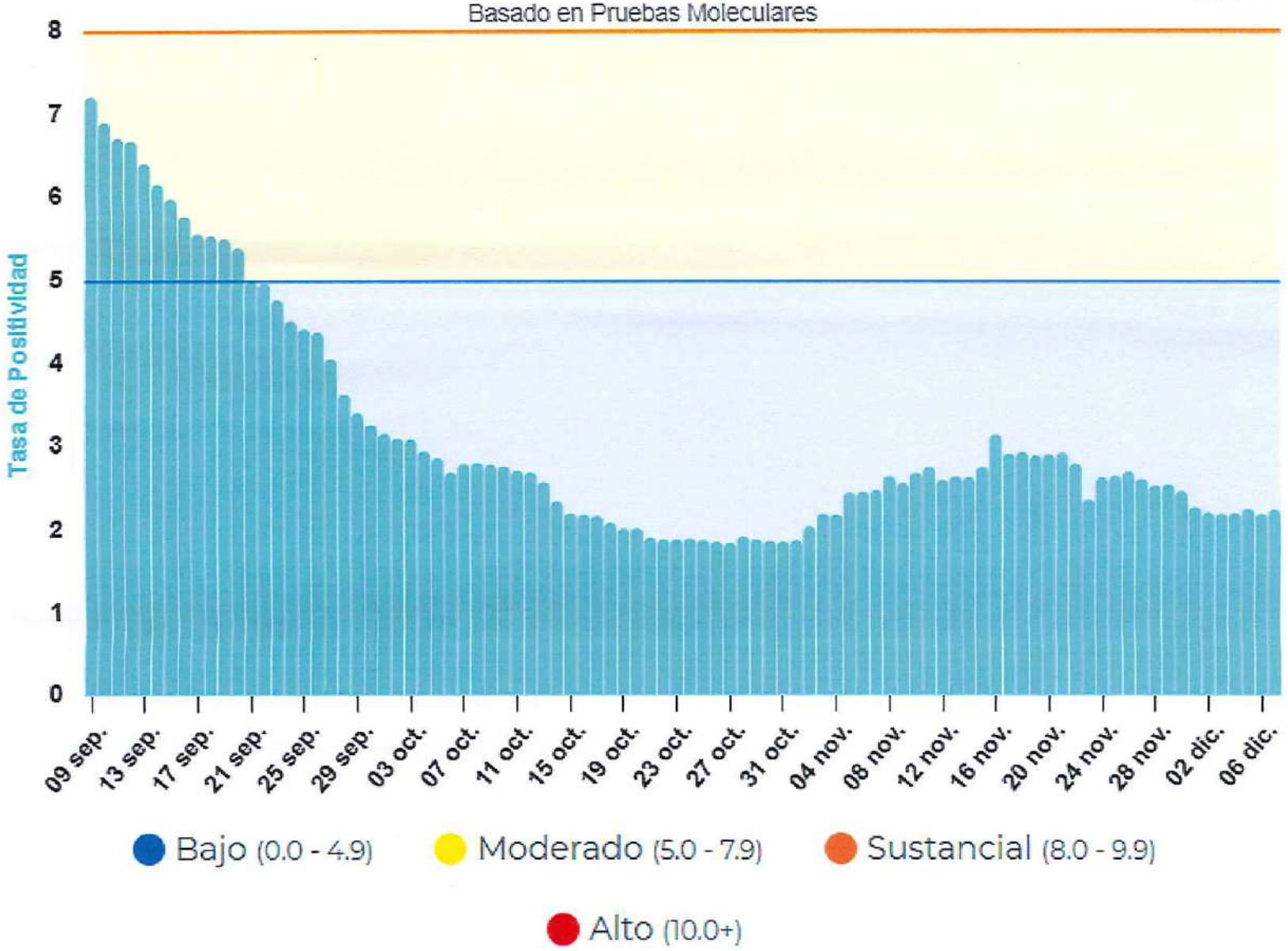
Conteo diario de casos confirmados (PCR) para COVID-19 notificadas por fecha de toma de muestra



Puerto Rico Health Department, COVID19 Dashboard, *Casos*, https://covid19datos.salud.gov.pr/#casos

Positividad

Basado en Pruebas Moleculares



Puerto Rico Health Department, COVID19 Dashboard, Resumen Ejecutivo, https://covid19datos.salud.gov.pr/#resumen_ejec

¿Quiénes se han vacunado?

11/03/2021 12/10/2021

12/02/2020 12/10/2021

Datos de Puerto Rico

Datos reportados al 11/12/2021

Personas aptas (5 años o más) con al menos una dosis	140,488 4.6% de 3,076,212

Manufacturero	Personas aptas (5 años o más) con al menos una dosis
Janssen	1,161
Moderna	28,783
Pfizer	110,544
Total	**140,488**

¿QUE VEO EN ESTE DIAGRAMA?

Personas aptas (5 años o más) con serie de vacunas completadas	76,155 2.5% de 3,076,212

¿QUE VEO EN ESTE DIAGRAMA?

Personas aptas (18 años o más) con dosis de refuerzo	262,275 10% de 2,620,963

Datos obtenidos del Puerto Rico Electronic Immunization System (PREIS)

¿QUE VEO EN ESTE DIAGRAMA?

Grupos de edad | Sexo

Grupo de edad	Personas aptas (5 años o más) con al menos una dosis de vacunas
5 - 11	79,132
12 - 15	2,197
16 - 19	1,076
20 - 29	6,547
30 - 39	6,943
40 - 49	7,751
50 - 59	9,679
60 - 69	11,006
70 - 79	9,939
80 +	6,098
No Definido	120
Total	**140,488**

Puerto Rico Health Department, COVID-19 en Cifras en Puerto Rico, *Vacunacion*, https://covid19datos.salud.gov.pr/#vacunacion

Data Table for Cumulative Cases per 100k in Last 7 Days

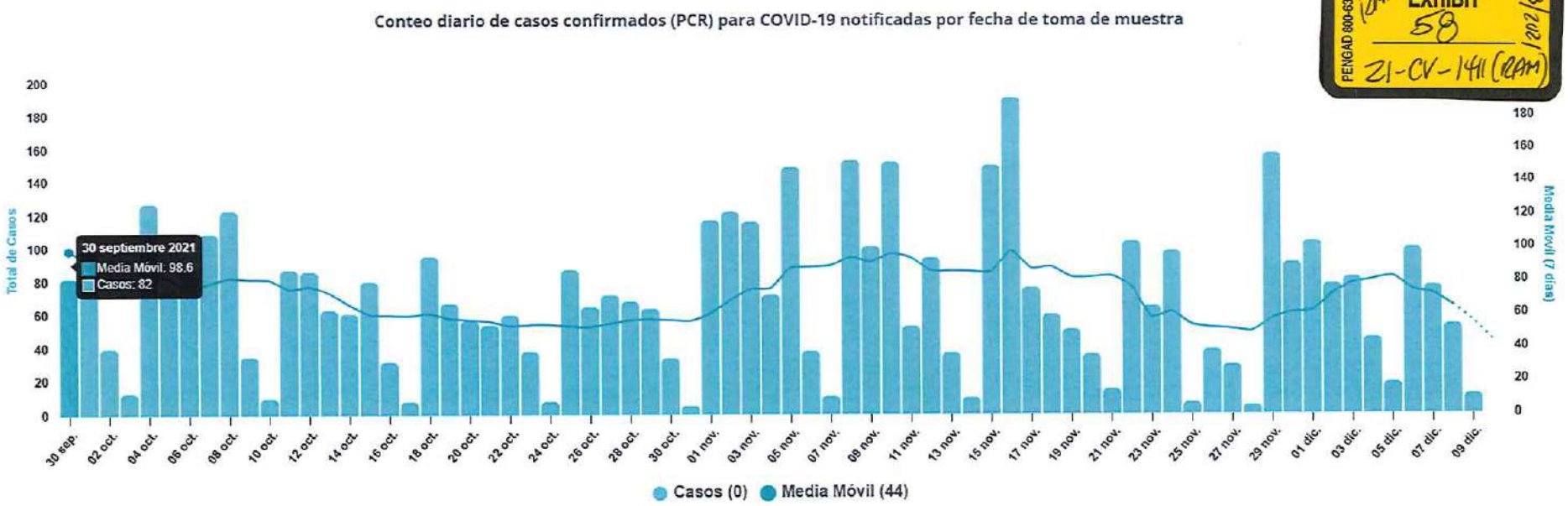
CDC | Data as of: November 15, 2021 12:40 PM ET. Posted: November 15, 2021 2:00 PM ET

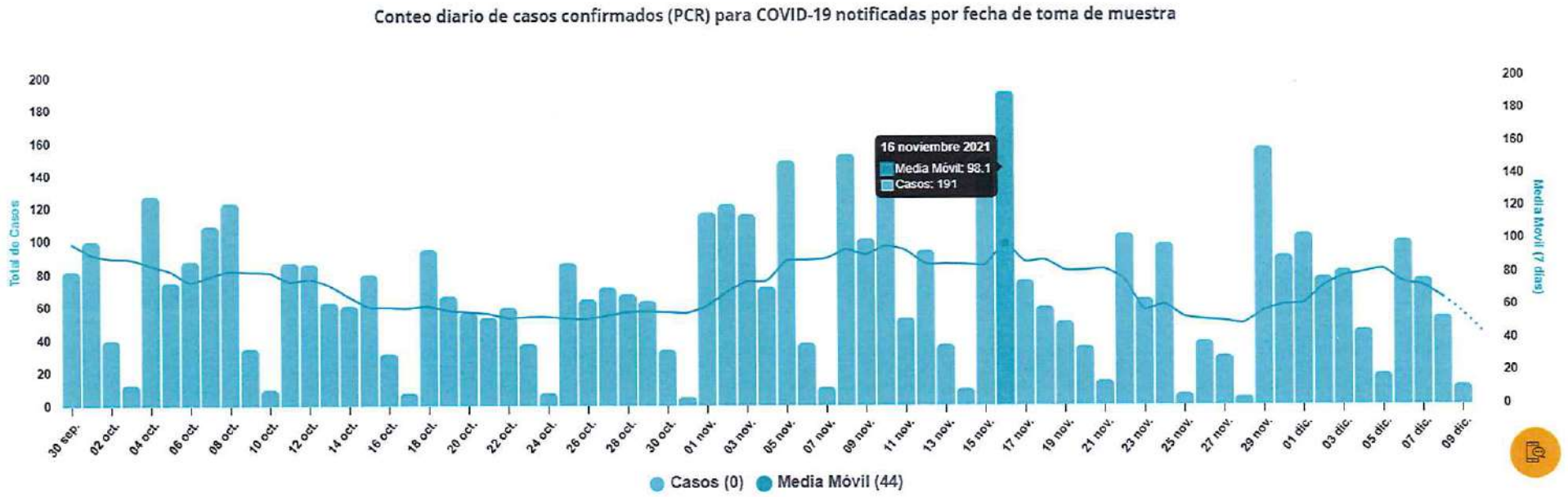
Download Data

State/Territory	7-Day Case Rate per 100,000
Michigan	503.8
Minnesota	490.2
New Mexico	461.7
North Dakota	437.2
New Hampshire	405.8
Wisconsin	395.8
Alaska	375
Vermont	367.5
Wyoming	365.1
Colorado	360.9
Montana	358
Utah	356.6
Arizona	341.1
Nebraska	312.8
South Dakota	304.3
Indiana	303.5
Ohio	283.1
Iowa	281.8
Kansas	280.5
West Virginia	278.1
New York*	276.3
Pennsylvania	267.7
Maine	251.2
Rhode Island	225.1
Kentucky	207.9
Delaware	206.4
New York (Level of Community Transmission)*	199.2
Massachusetts	193.3
Illinois	190.7
Idaho	189.4
Missouri	160.8
Washington	158.8
Nevada	148.6
Oklahoma	141.1
Oregon	138.4
Guam	137.1
New Jersey	128.9
Tennessee	124.7
North Carolina	117.3
Arkansas	116.8
Connecticut	112.7
Virginia	104.2
Northern Mariana Islands	102.2
Maryland	98.6
New York City*	97.6
California	93.1
District of Columbia	81.2
Georgia	79.9
Texas	73.8
Mississippi	72.5
Alabama	66.2
Virgin Islands	63
Louisiana	56.7
Florida	51
Hawaii	49.4
South Carolina	41.6
Puerto Rico	26.7
Palau	0
Republic of Marshall Islands	0
American Samoa	N/A
Federated States of Micronesia	N/A

Footnotes

CDC, United States COVID-19 Cases, Deaths, and Laboratory Testing (NAATs) by State, Territory, and Jurisdiction, (View: Cases, Time Period: Last 7 Days, Metric: Rate per 100,000, *Data Table for Cumulative Cases per 100k in Last 7 Days*, https://web.archive.org/web/20211116192651/https://covid.cdc.gov/covid-data-tracker/#cases_casesper100klast7days





Puerto Rico Health Department, COVID-19 en Cifras en Puerto Rico, *Casos*, https://covid19datos.salud.gov.pr/#casos

New Admissions of Patients with Confirmed COVID-19, Puerto Rico

Aug 01, 2020 - Dec 08, 2021

13,566
Total Admissions
Aug 01, 2020 - Dec 08, 2021

4
Current 7-Day Average
Dec 02, 2021 - Dec 08, 2021

3
Prior 7-Day Average
Nov 25, 2021 - Dec 01, 2021

189
Peak 7-Day Average
Nov 04, 2020 - Nov 10, 2020

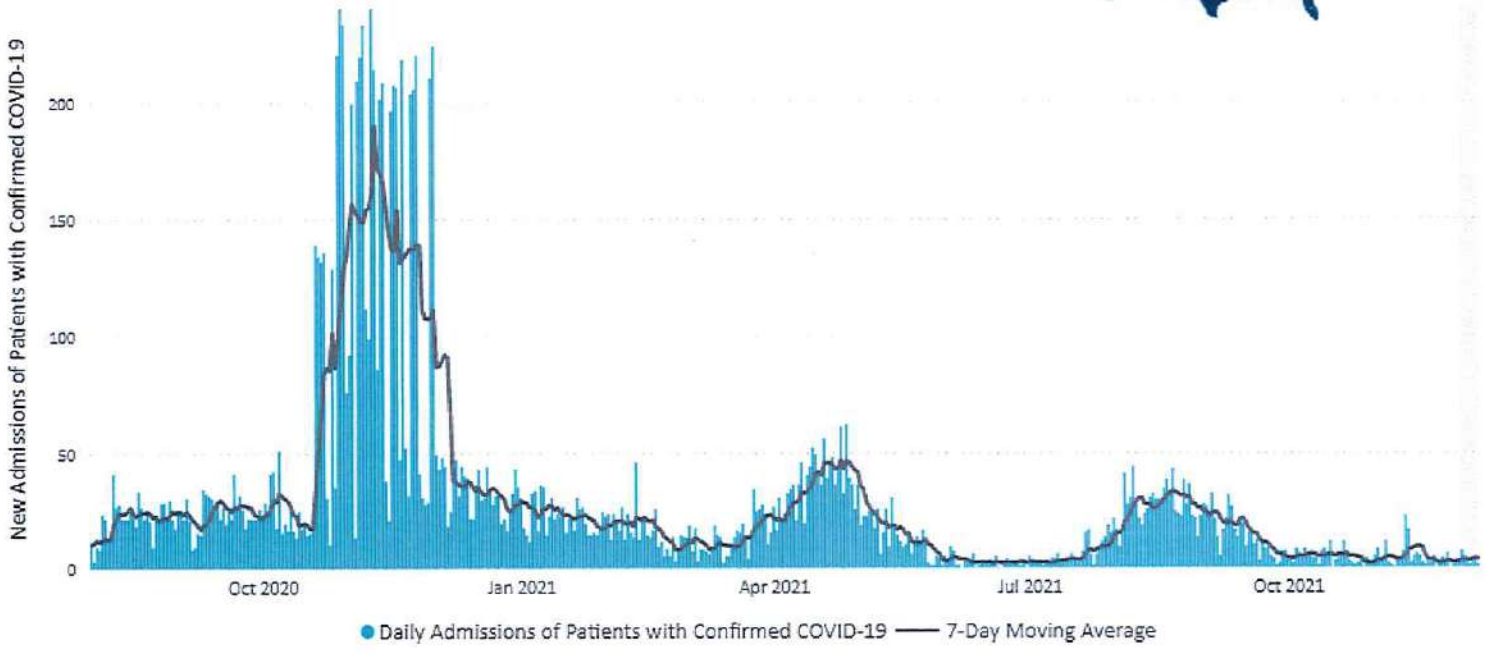
+31.6%
Percent change from prior 7-day avg. of Nov 25, 2021 - Dec 01, 2021

-98.1%
Percent change from peak 7-day avg. of Nov 04, 2020 - Nov 10, 2020

By Jurisdiction and Age Group | By Jurisdiction

Select a Jurisdiction: Puerto Rico

HHS Regions

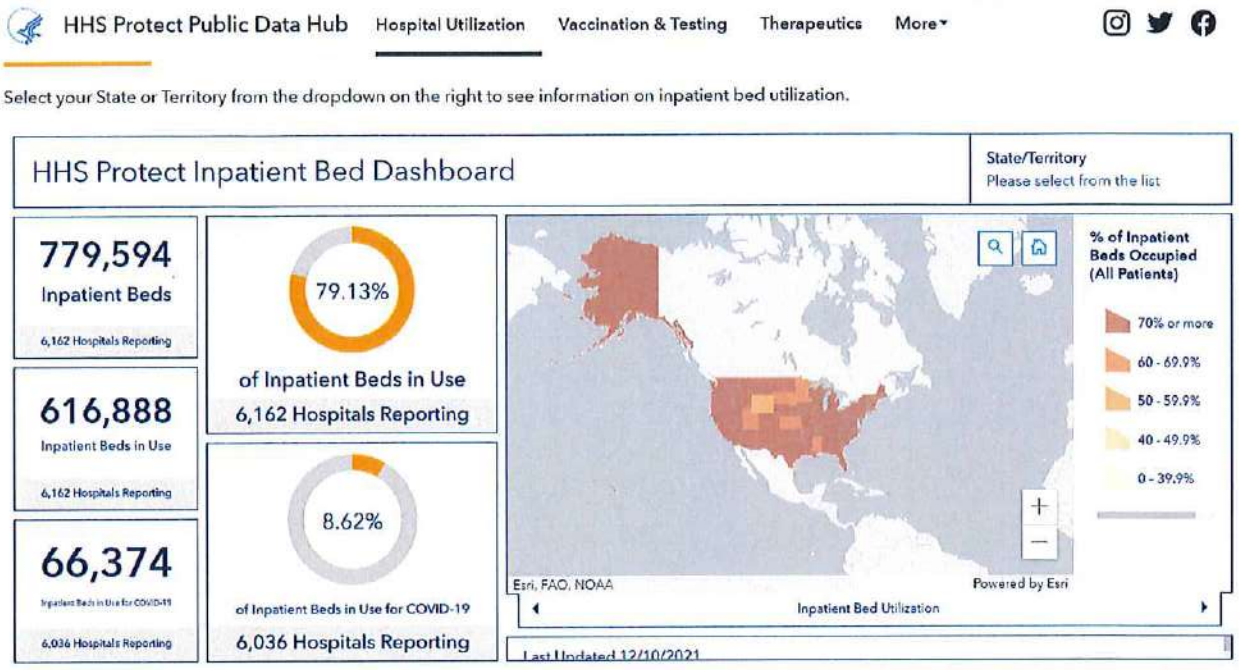


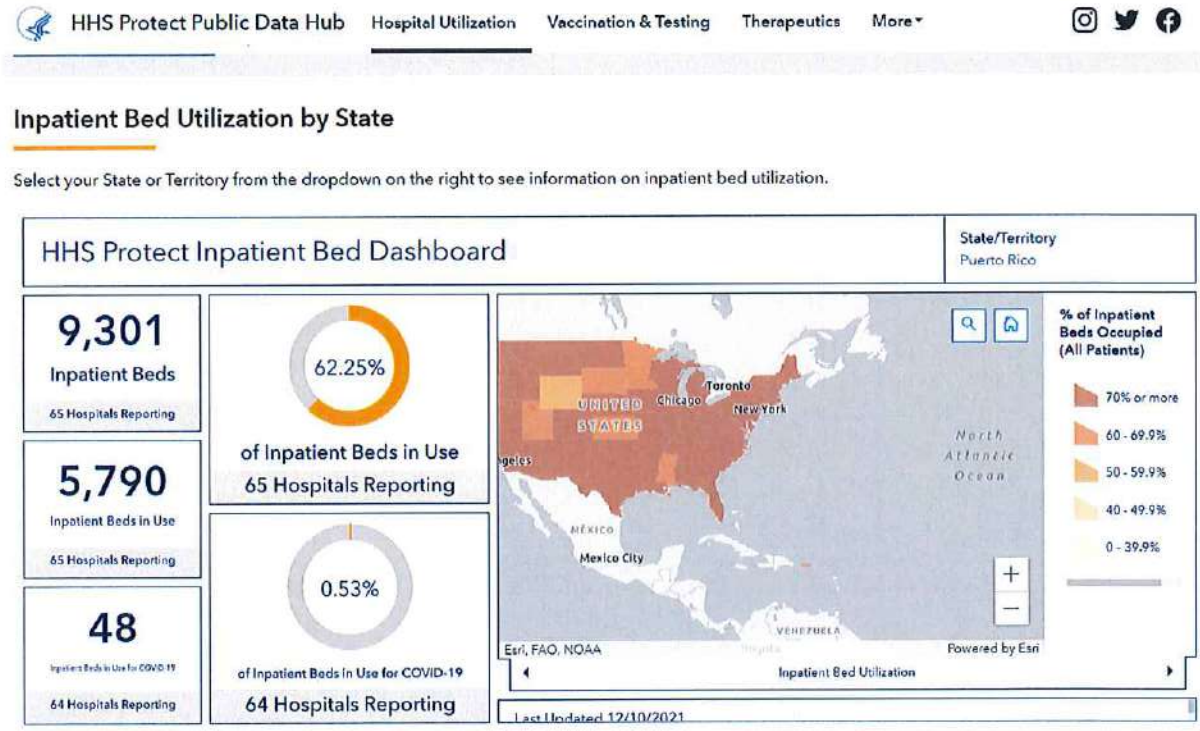
Based on reporting from all hospitals (N=5,260). Due to potential reporting delays, data reported in the most recent 7 days (as represented by the shaded bar) should be interpreted with caution.
Small shifts in historic data may occur due to changes in the CMS Provider of Services file, which is used to identify the cohort of included hospitals. Data since December 1, 2020 have had error correction methodology applied. Data prior to this date may have anomalies that are still being resolved. Data prior to August 1, 2020 are unavailable.

Last Updated: Dec 10, 2021

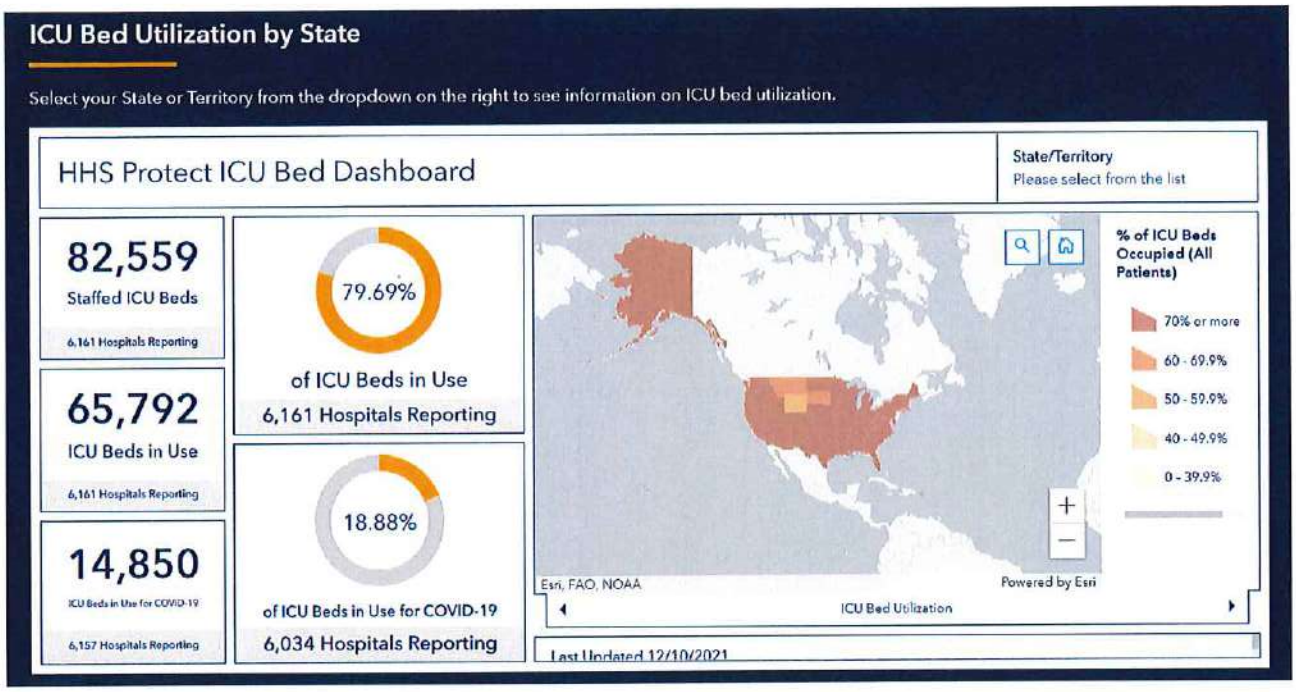
Unified Hospital Dataset, White House COVID-19 Team, Data Strategy and Execution Workgroup

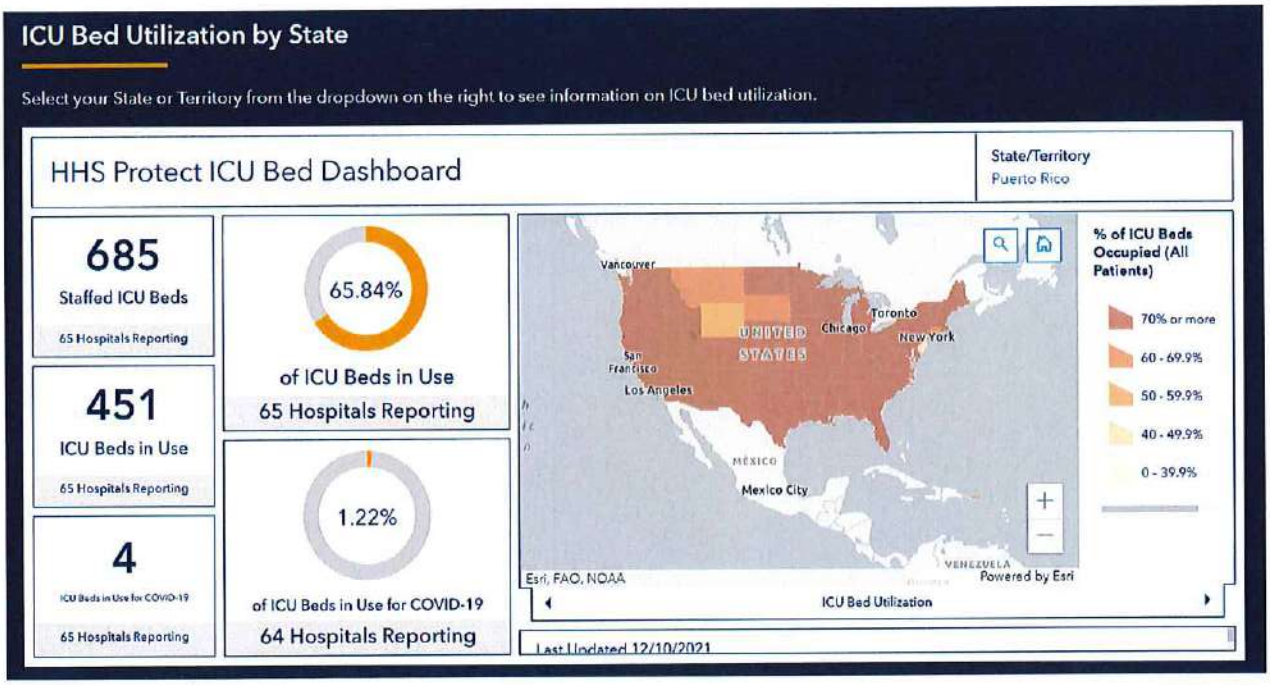
CDC, New Hospital Admissions, *New Admissions of Patients with Confirmed COVID-19, Puerto Rico* (By Jurisdiction, Select Jurisdiction: Puerto Rico), https://covid.cdc.gov/covid-data-tracker/#new-hospital-admissions.





HHS, *HHS Protect Inpatient Bed Dashboard*, https://protect-public.hhs.gov/pages/hospital-utilization





HHS, *HHS Protect ICU Bed Dashboard*, https://protect-public.hhs.gov/pages/hospital-utilization

Data Table for Cumulative Deaths per 100k in Last 7 Days

CDC | Data as of: November 15, 2021 12:40 PM ET. Posted: November 15, 2021 2:00 PM ET

Download Data

State/Territory	7-Day Death Rate per 100,000
Wyoming	9.5
Montana	6.3
Alaska	5.3
Kentucky	4.9
Ohio	4.9
West Virginia	4.9
Oregon	4.3
Idaho	4.1
Pennsylvania	3.9
Georgia	3.7
Colorado	3.5
North Dakota	3.5
Arizona	3.4
New Mexico	3.2
Iowa	3.1
Guam	3
Texas	3
Kansas	2.9
Indiana	2.8
Nevada	2.8
Alabama	2.5
South Dakota	2.5
Arkansas	2.4
Minnesota	2.4
Delaware	2.3
New Hampshire	2.3
Utah	2.3
Virginia	2.3
Michigan	2.2
Wisconsin	2.2
Maine	2.1
Washington	2.1
Oklahoma	1.9
Tennessee	1.9
Louisiana	1.7
Nebraska	1.6
New York*	1.6
California	1.5
Missouri	1.5
Massachusetts	1.4
Maryland	1.3
Mississippi	1.3
Rhode Island	1.3
Illinois	1.2
Hawaii	1.1
North Carolina	1.1
Vermont	1.1
Connecticut	1
New Jersey	1
New York City*	0.9
South Carolina	0.9
Virgin Islands	0.9
District of Columbia	0.3
Puerto Rico	0.3
Florida	0.1
Northern Mariana Islands	0
Palau	0
Republic of Marshall Islands	0
American Samoa	N/A
Federated States of Micronesia	N/A
New York (Level of Community Transmission)*	N/A

Footnotes

CDC, United States COVID-19 Cases, Deaths, and Laboratory Testing (NAATs) by State, Territory, and Jurisdiction, (View: Deaths, Time Period: Last 7 Days, Metric: Rate per 100,000), *Data Table for Cumulative Cases per 100k in Last 7 Days*, https://web.archive.org/web/20211116192651/https://covid.cdc.gov/covid-data-tracker/#cases_deathsper100klast7days

Data Table for Cumulative Deaths per 100k in Last 7 Days

CDC | Data as of: December 10, 2021 12:55 PM ET. Posted: December 10, 2021 3:36 PM ET

Download Data

State/Territory	7-Day Death Rate per 100,000
Wyoming	7.6
Arizona	6.5
West Virginia	6.1
Tennessee	5.9
Pennsylvania	4.9
Michigan	4.7
Minnesota	4.6
Montana	4.6
Indiana	4.5
North Dakota	4.2
South Dakota	4.2
Nebraska	4.1
Wisconsin	4
Arkansas	3.9
Colorado	3.8
Idaho	3.6
Ohio	3.6
Iowa	3.3
Nevada	3.3
New Mexico	3.2
Kentucky	3
New Hampshire	3
Oregon	3
Delaware	2.9
Utah	2.7
Illinois	2.5
Maine	2.5
New York*	2.4
Kansas	2.2
Massachusetts	2.1
Washington	2
Georgia	1.8
Missouri	1.8
Virginia	1.7
South Carolina	1.5
Texas	1.5
North Carolina	1.4
Vermont	1.4
California	1.3
New Jersey	1.2
Louisiana	1.1
New York City*	1.1
Rhode Island	1.1
Connecticut	1
Hawaii	0.9
Alabama	0.8
Mississippi	0.7
Guam	0.6
Alaska	0.4
Maryland	0.3
Florida	0.1
Oklahoma	0.1
Puerto Rico	0.1
District of Columbia	0
Northern Mariana Islands	0
Palau	0
Republic of Marshall Islands	0
Virgin Islands	0
American Samoa	N/A
Federated States of Micronesia	N/A
New York (Level of Community Transmission)*	N/A

Footnotes

CDC, United States COVID-19 Cases, Deaths, and Laboratory Testing (NAATs) by State, Territory, and Jurisdiction, (View: Deaths, Time Period: Last 7 Days, Metric: Rate per 100,000), *Data Table for Cumulative Cases per 100k in Last 7 Days*, https://covid.cdc.gov/covid-data-tracker/#cases_deathsper100klast7days

Community transmission and viral load kinetics of the SARS-CoV-2 delta (B.1.617.2) variant in vaccinated and unvaccinated individuals in the UK: a prospective, longitudinal, cohort study

Anika Singanayagam*, Seran Hakki*, Jake Dunning*, Kieran J Madon, Michael A Crone, Aleksandra Koycheva, Nieves Derqui-Fernandez, Jack L Barnett, Michael G Whitfield, Robert Varro, Andre Charlett, Rhia Kundu, Joe Fenn, Jessica Cutajar, Valerie Quinn, Emily Conibear, Wendy Barclay, Paul S Freemont, Graham P Taylor, Shazaad Ahmad, Maria Zambon, Neil M Ferguson†, Ajit Lalvani†, on behalf of the ATACCC Study Investigators‡

Summary

Background The SARS-CoV-2 delta (B.1.617.2) variant is highly transmissible and spreading globally, including in populations with high vaccination rates. We aimed to investigate transmission and viral load kinetics in vaccinated and unvaccinated individuals with mild delta variant infection in the community.

Methods Between Sept 13, 2020, and Sept 15, 2021, 602 community contacts (identified via the UK contract-tracing system) of 471 UK COVID-19 index cases were recruited to the Assessment of Transmission and Contagiousness of COVID-19 in Contacts cohort study and contributed 8145 upper respiratory tract samples from daily sampling for up to 20 days. Household and non-household exposed contacts aged 5 years or older were eligible for recruitment if they could provide informed consent and agree to self-swabbing of the upper respiratory tract. We analysed transmission risk by vaccination status for 231 contacts exposed to 162 epidemiologically linked delta variant-infected index cases. We compared viral load trajectories from fully vaccinated individuals with delta infection (n=29) with unvaccinated individuals with delta (n=16), alpha (B.1.1.7; n=39), and pre-alpha (n=49) infections. Primary outcomes for the epidemiological analysis were to assess the secondary attack rate (SAR) in household contacts stratified by contact vaccination status and the index cases' vaccination status. Primary outcomes for the viral load kinetics analysis were to detect differences in the peak viral load, viral growth rate, and viral decline rate between participants according to SARS-CoV-2 variant and vaccination status.

Findings The SAR in household contacts exposed to the delta variant was 25% (95% CI 18–33) for fully vaccinated individuals compared with 38% (24–53) in unvaccinated individuals. The median time between second vaccine dose and study recruitment in fully vaccinated contacts was longer for infected individuals (median 101 days [IQR 74–120]) than for uninfected individuals (64 days [32–97], p=0·001). SAR among household contacts exposed to fully vaccinated index cases was similar to household contacts exposed to unvaccinated index cases (25% [95% CI 15–35] for vaccinated *vs* 23% [15–31] for unvaccinated). 12 (39%) of 31 infections in fully vaccinated household contacts arose from fully vaccinated epidemiologically linked index cases, further confirmed by genomic and virological analysis in three index case–contact pairs. Although peak viral load did not differ by vaccination status or variant type, it increased modestly with age (difference of 0·39 [95% credible interval –0·03 to 0·79] in peak $\log_{10}$ viral load per mL between those aged 10 years and 50 years). Fully vaccinated individuals with delta variant infection had a faster (posterior probability >0·84) mean rate of viral load decline (0·95 $\log_{10}$ copies per mL per day) than did unvaccinated individuals with pre-alpha (0·69), alpha (0·82), or delta (0·79) variant infections. Within individuals, faster viral load growth was correlated with higher peak viral load (correlation 0·42 [95% credible interval 0·13 to 0·65]) and slower decline (–0·44 [–0·67 to –0·18]).

Interpretation Vaccination reduces the risk of delta variant infection and accelerates viral clearance. Nonetheless, fully vaccinated individuals with breakthrough infections have peak viral load similar to unvaccinated cases and can efficiently transmit infection in household settings, including to fully vaccinated contacts. Host–virus interactions early in infection may shape the entire viral trajectory.

Funding National Institute for Health Research.

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See Online/Comment https://doi.org/10.1016/S1473-3099(21)00690-3

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Introduction

While the primary aim of vaccination is to protect individuals against severe COVID-19 disease and its consequences, the extent to which vaccines reduce onward transmission of SARS-CoV-2 is key to containing the pandemic. This outcome depends on the ability of

London Biofoundry, Imperial College Translation and Innovation Hub, London, UK (M A Crone, Prof P S Freemont); National Infection Service (A Singanayagam, J Dunning, Prof M Zambon FRCPath) and Data and Analytical Services (A Charlett PhD), Public Health England, London, UK; Department of Virology, Manchester Medical Microbiology Partnership, Manchester Foundation Trust, Manchester Academic Health Sciences Centre, Manchester, UK (S Ahmad FRCPath)

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Research in context

Evidence before this study

The SARS-CoV-2 delta variant is spreading globally, including in populations with high vaccination coverage. While vaccination remains highly effective at attenuating disease severity and preventing death, vaccine effectiveness against infection is reduced for delta. Determining the extent of transmission from vaccinated delta-infected individuals to their vaccinated contacts is a public health priority. Comparing the upper respiratory tract (URT) viral load kinetics of delta infections with those of other variants gives insight into potential mechanisms for its increased transmissibility. We searched PubMed and *medRxiv* for articles published between database inception and Sept 20, 2021, using search terms describing "SARS-CoV-2, delta variant, viral load, and transmission". Two studies longitudinally sampled the URT in vaccinated and unvaccinated delta variant-infected individuals to compare viral load kinetics. In a retrospective study of a cohort of hospitalised patients in Singapore, more rapid viral load decline was found in vaccinated individuals than unvaccinated cases. However, the unvaccinated cases in this study had moderate-to-severe infection, which is known to be associated with prolonged shedding. The second study longitudinally sampled professional USA sports players. Again, clearance of delta viral RNA in vaccinated cases was faster than in unvaccinated cases, but only 8% of unvaccinated cases had delta variant infection, complicating interpretation. Lastly, a report of a single-source nosocomial outbreak of a distinct delta sub-lineage in Vietnamese health-care workers plotted viral load kinetics (without comparison with unvaccinated delta infections) and demonstrated transmission between fully vaccinated health-care workers in the nosocomial setting. The findings might therefore not be generalisable beyond the particular setting and distinct viral sub-lineage investigated.

Added value of this study

The majority of SARS-CoV-2 transmission occurs in households, but transmission between fully vaccinated individuals in this setting has not been shown to date. To ascertain secondary transmission with high sensitivity, we longitudinally followed index cases and their contacts (regardless of symptoms) in the community early after exposure to the delta variant of SARS-CoV-2, performing daily quantitative RT-PCR on URT samples for 14–20 days. We found that the secondary attack rate in fully vaccinated household contacts was high at 25%, but this value was lower than that of unvaccinated contacts (38%). Risk of infection increased with time in the 2–3 months since the second dose of vaccine. The proportion of infected contacts was similar regardless of the index cases' vaccination status. We observed transmission of the delta variant between fully vaccinated index cases and their fully vaccinated contacts in several households, confirmed by whole-genome sequencing. Peak viral load did not differ by vaccination status or variant type but did increase modestly with age. Vaccinated delta cases experienced faster viral load decline than did unvaccinated alpha or delta cases. Across study participants, faster viral load growth was correlated with higher peak viral load and slower decline, suggesting that host–virus interactions early in infection shape the entire viral trajectory. Since our findings are derived from community household contacts in a real-life setting, they are probably generalisable to the general population.

Implications of all the available evidence

Although vaccines remain highly effective at preventing severe disease and deaths from COVID-19, our findings suggest that vaccination is not sufficient to prevent transmission of the delta variant in household settings with prolonged exposures. Our findings highlight the importance of community studies to characterise the epidemiological phenotype of new SARS-CoV-2 variants in increasingly highly vaccinated populations. Continued public health and social measures to curb transmission of the delta variant remain important, even in vaccinated individuals.

vaccines to protect against infection and the extent to which vaccination reduces the infectiousness of breakthrough infections.

Vaccination was found to be effective in reducing household transmission of the alpha variant (B.1.1.7) by 40–50%,[1] and infected, vaccinated individuals had lower viral load in the upper respiratory tract (URT) than infections in unvaccinated individuals,[2] which is indicative of reduced infectiousness.[3,4] However, the delta variant (B.1.617.2), which is more transmissible than the alpha variant,[5,6] is now the dominant strain worldwide. After a large outbreak in India, the UK was one of the first countries to report a sharp rise in delta variant infection. Current vaccines remain highly effective at preventing admission to hospital and death from delta infection.[7] However, vaccine effectiveness against infection is reduced for delta, compared with alpha,[8,9] and the delta variant continues to cause a high burden of cases even in countries with high vaccination coverage. Data are scarce on the risk of community transmission of delta from vaccinated individuals with mild infections.

Here, we report data from a UK community-based study, the Assessment of Transmission and Contagiousness of COVID-19 in Contacts (ATACCC) study, in which ambulatory close contacts of confirmed COVID-19 cases underwent daily, longitudinal URT sampling, with collection of associated clinical and epidemiological data. We aimed to quantify household transmission of the delta variant and assess the effect of vaccination status on contacts' risk of infection and index cases' infectiousness, including (1) households with unvaccinated contacts and index cases and (2) households with fully vaccinated contacts and fully vaccinated index cases. We also compared sequentially sampled

URT viral RNA trajectories from individuals with non-severe delta, alpha, and pre-alpha SARS-CoV-2 infections to infer the effects of SARS-CoV-2 variant status—and, for delta infections, vaccination status—on transmission potential.

Methods

Study design and participants

ATACCC is an observational longitudinal cohort study of community contacts of SARS-CoV-2 cases. Contacts of symptomatic PCR-confirmed index cases notified to the UK contact-tracing system (National Health Service Test and Trace) were asked if they would be willing to be contacted by Public Health England to discuss participation in the study. All contacts notified within 5 days of index case symptom onset were selected to be contacted within our recruitment capacity. Household and non-household contacts aged 5 years or older were eligible for recruitment if they could provide written informed consent and agree to self-swabbing of the URT. Further details on URT sampling are given in the appendix (p 13).

The ATACCC study is separated into two study arms, ATACCC1 and ATACCC2, which were designed to capture different waves of the SARS-CoV-2 pandemic. In ATACCC1, which investigated alpha variant and pre-alpha cases in Greater London, only contacts were recruited between Sept 13, 2020, and March 13, 2021. ATACCC1 included a pre-alpha wave (September to November, 2020) and an alpha wave (December, 2020, to March, 2021). In ATACCC2, the study was relaunched specifically to investigate delta variant cases in Greater London and Bolton, and both index cases and contacts were recruited between May 25, and Sept 15, 2021. Early recruitment was focused in West London and Bolton because UK incidence of the delta variant was highest in these areas.[10] Based on national and regional surveillance data, community transmission was moderate-to-high throughout most of our recruitment period.

This study was approved by the Health Research Authority. Written informed consent was obtained from all participants before enrolment. Parents and caregivers gave consent for children.

Data collection

Demographic information was collected by the study team on enrolment. The date of exposure for non-household contacts was obtained from Public Health England. COVID-19 vaccination history was determined from the UK National Immunisation Management System, general practitioner records, and self-reporting by study participants. We defined a participant as unvaccinated if they had not received a single dose of a COVID-19 vaccine at least 7 days before enrolment, partially vaccinated if they had received one vaccine dose at least 7 days before study enrolment, and fully vaccinated if they had received two doses of a COVID-19 vaccine at least 7 days before study enrolment. Previous literature was used to determine the 7-day threshold for defining vaccination status.[11–13] We also did sensitivity analyses using a 14-day threshold. The time interval between vaccination and study recruitment was calculated. We used WHO criteria[14] to define symptomatic status up to the day of study recruitment. Symptomatic status for incident cases—participants who were PCR-negative at enrolment and subsequently tested positive—was defined from the day of the first PCR-positive result.

Laboratory procedures

SARS-CoV-2 quantitative RT-PCR, conversion of ORF1ab and envelope (E-gene) cycle threshold values to viral genome copies, whole-genome sequencing, and lineage assignments are described in the appendix (pp 13–14).

Outcomes

Primary outcomes for the epidemiological analysis were to assess the secondary attack rate (SAR) in household contacts stratified by contact vaccination status and the index cases' vaccination status. Primary outcomes for the viral load kinetics analysis were to detect differences in the peak viral load, viral growth rate, and viral decline rate between participants infected with pre-alpha versus alpha versus delta variants and between unvaccinated delta-infected participants and vaccinated delta-infected participants.

See Online for appendix

We assessed vaccine effectiveness and susceptibility to SARS-CoV-2 infection stratified by time elapsed since receipt of second vaccination as exploratory analyses.

Statistical analysis

To model viral kinetics, we used a simple phenomenological model of viral titre[15] during disease pathogenesis. Viral kinetic parameters were estimated on a participant-specific basis using a Bayesian hierarchical model to fit this model to the entire dataset of sequential cycle threshold values measured for all participants. For the 19 participants who were non-household contacts of index cases and had a unique date of exposure, the cycle threshold data were supplemented by a pseudo-absence data point (ie, undetectable virus) on the date of exposure. Test accuracy and model misspecification were modelled with a mixture model by assuming there was a probability p of a test giving an observation drawn from a (normal) error distribution and probability $1-p$ of it being drawn from the true distribution.

The hierarchical structure was represented by grouping participants based on the infecting variant and their vaccination status. A single-group model was fitted, which implicitly assumes that viral kinetic parameters vary by individual but not by variant or vaccination status. A four-group model was also explored, where groups 1, 2, 3, and 4 represent pre-alpha, alpha, unvaccinated delta, and fully vaccinated delta, respectively. We fitted a correlation matrix between

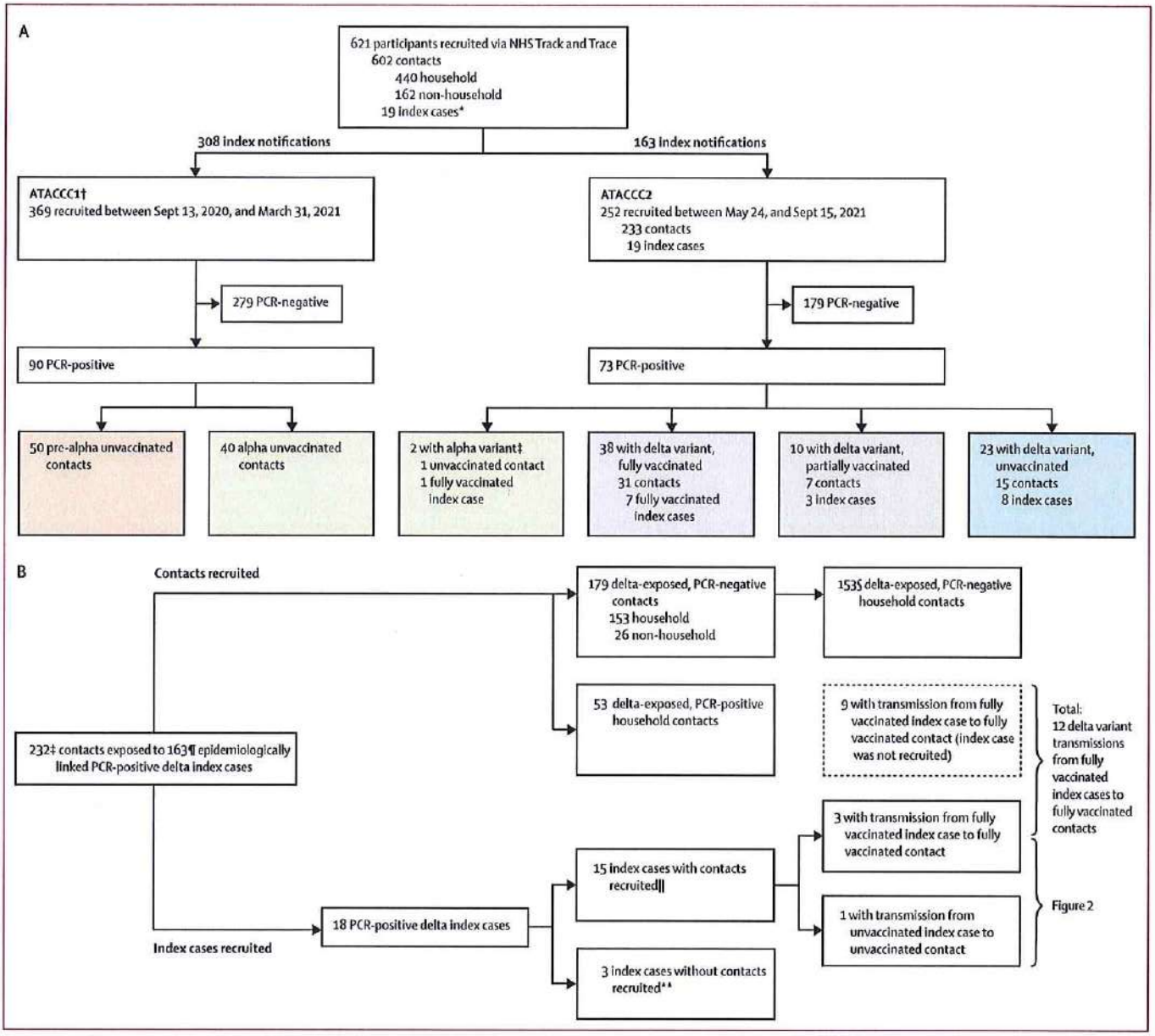


Figure 1: **Recruitment, SARS-CoV-2 infection, variant status, and vaccination history for ATACCC study participants**
(A) Study recruitment and variant status confirmed by whole-genome sequencing (ATACCC1 and ATACCC2 combined). (B) ATACCC2: delta-exposed contacts included in secondary attack rate calculation (table 1) and transmission assessment (table 2). NHS=National Health Service. *All index cases were from ATACCC2. †All contacts. ‡The two earliest PCR-positive cases from the ATACCC2 cohort (one index case and one contact) were confirmed as having the alpha variant on whole-genome sequencing (recruited on May 28, 2021). This alpha variant-exposed, PCR-positive contact is excluded from figure 1B. §One PCR-negative contact had no vaccination status data available and one PCR-negative contact's index case had no vaccination data available. ¶Vaccination data were available for 138 index cases of 163. ||The contacts of these 15 index cases are included within the 232 total contacts. **These three index cases without contacts are only included in the viral load kinetics analysis (figure 3) and are not included in tables 1 and 2.

participant-specific kinetic parameters to allow us to examine whether there is within-group correlation between peak viral titre, viral growth rate, and viral decline rate. Our initial model selection, using leave-one-out cross-validation, selected a four-group hierarchical model with fitted correlation coefficients between individual-level parameters determining peak viral load and viral load growth and decline rates (appendix p 5). However, resulting participant-specific estimates of peak viral load (but not growth and decline rates) showed a marked and significant correlation with age in the exploratory analysis, which motivated examination of models where mean peak viral load could vary with age. The most predictive model overall allowed mean viral

load growth and decline rates to vary across the four groups, with mean peak viral load common to all groups but assumed to vary linearly with the logarithm of age (appendix p 5). We present peak viral loads for the reference age of 50 years with 95% credible intervals (95% CrIs). 50 years was chosen as the reference age as it is typical of the ages of the cases in the whole dataset and the choice of reference age made no difference in the model fits or judgment of differences between the groups.

We computed group-level population means and within-sample group means of log peak viral titre, viral growth rate, and viral decline rate. Since posterior estimates of each of these variables are correlated across groups, overlap in the credible intervals of an estimate for one group with that for another group does not necessarily indicate no significant difference between those groups. We, therefore, computed posterior probabilities, *pp*, that these variables were larger for one group than another. For our model, Bayes factors can be computed as $pp/(1-pp)$. We only report population (group-level) posterior probabilities greater than 0·75 (corresponding to Bayes factors >3) as indicating at least moderate evidence of a difference.

For vaccine effectiveness, we defined the estimated effectiveness at preventing infection, regardless of symptoms, with delta in the household setting as 1 – SAR (fully vaccinated) / SAR (unvaccinated).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Sept 13, 2020, and Sept 15, 2021, 621 community-based participants (602 contacts and 19 index cases) from 471 index notifications were prospectively enrolled in the ATACCC1 and ATACCC2 studies, and contributed 8145 URT samples. Of these, ATACCC1 enrolled 369 contacts (arising from 308 index notifications), and ATACCC2 enrolled 233 contacts (arising from 163 index notifications) and 19 index cases. SARS-CoV-2 RNA was detected in 163 (26%) of the 621 participants. Whole-genome sequencing of PCR-positive cases confirmed that 71 participants had delta variant infection (18 index cases and 53 contacts), 42 had alpha variant infection (one index case and 41 contacts), and 50 had pre-alpha variant infection (all contacts; figure 1A).

Of 163 PCR-positive participants, 89 (55%) were female and 133 (82%) were White. Median age was 36 years (IQR 26–50). Sex, age, ethnicity, body-mass index (BMI) distribution, and the frequency of comorbidities were similar among those with delta, alpha, and pre-alpha infection, and for vaccinated and unvaccinated delta-infected participants, except for age and sex (appendix pp 2–3). There were fewer unvaccinated females than males (p=0·04) and, as expected from the age-prioritisation of the UK vaccine roll-out, unvaccinated participants infected with the delta variant were significantly younger (p<0·001; appendix p 3). Median time between exposure to the index case and study enrolment was 4 days (IQR 4–5). All participants had non-severe ambulatory illness or were asymptomatic. The proportion of asymptomatic cases did not differ among fully vaccinated, partially vaccinated, and unvaccinated delta groups (appendix p 3).

	Total	PCR positive	PCR negative	SAR (95% CI)	p value
Contacts					
All	231	53	178	23 (18-29)	NA
Fully vaccinated	140	31	109	22 (16-30)	0·16
Unvaccinated	44	15	29	34 (22-49)	..
Partially vaccinated	47	7	40	15 (7-28)	NA
Household contacts					
All	205	53	152	26 (20-32)	NA
Fully vaccinated	126	31	95	25 (18-33)	0·17
Unvaccinated	40	15	25	38 (24-53)	..
Partially vaccinated	39	7	32	18 (9-33)	NA

χ^2 test was performed to calculate p values for differences in SAR between fully vaccinated and unvaccinated cases. One PCR-negative contact who withdrew from the study without vaccination status information was excluded. NA=not applicable. SAR=secondary attack rate.

Table 1: SAR in contacts of delta-exposed index cases recruited to the ATACCC2 study

No pre-alpha-infected and only one alpha-infected participant had received a COVID-19 vaccine before study enrolment. Of 71 delta-infected participants (of whom 18 were index cases), 23 (32%) were unvaccinated, ten (14%) were partially vaccinated, and 38 (54%) were fully vaccinated (figure 1A; appendix p 3). Of the 38 fully vaccinated delta-infected participants, 14 had received the BNT162b2 mRNA vaccine (Pfizer–BioNTech), 23 the ChAdOx1 nCoV-19 adenovirus vector vaccine (Oxford–AstraZeneca), and one the CoronaVac inactivated whole-virion vaccine (Sinovac).

It is highly probable that all but one of the 233 ATACCC2 contacts were exposed to the delta variant because they were recruited when the regional prevalence of delta was at least 90%, and mostly 95–99% (figure 1B).[10] Of these, 206 (89%) were household contacts (in 127 households), and 26 (11%) were non-household contacts. Distributions of age, ethnicity, BMI, smoking status, and comorbidities were similar between PCR-positive and PCR-negative contacts (appendix p 4). The median time between second vaccine dose and study recruitment in fully vaccinated contacts with delta variant infection was 74 days (IQR 35–105; range 16–201), and this was significantly longer in PCR-positive contacts than in PCR-negative contacts (101 days [IQR 74–120] *vs* 64 days [32–97], respectively, p=0·001; appendix p 4). All 53 PCR-positive contacts were exposed in household settings and the SAR for all delta variant-exposed household contacts was 26% (95% CI 20–32). SAR was

	All household contacts (n=204)*	Fully vaccinated contacts (n=125)		Partially vaccinated contacts (n=39)		Unvaccinated contacts (n=40)	
		PCR positive (n=31)	PCR negative (n=94)	PCR positive (n=7)	PCR negative (n=32)	PCR positive (n=15)	PCR negative (n=25)
Fully vaccinated index cases (n=50)	69	12	31	1	8	4	13
Partially vaccinated index cases (n=25)	35	7	12	3	10	3	0
Unvaccinated index cases (n=63)	100	12	51	3	14	8	12

Non-household exposed contacts (n=24, all PCR negative) were excluded. One PCR-negative household contact who withdrew from the study without vaccination status information was excluded. One PCR-negative household contact who could not be linked to their index case was also excluded. *The rows below show the number of contacts exposed to each category of index case.

Table 2: **Comparison of vaccination status of the 138 epidemiologically linked PCR-positive index cases for 204 delta variant-exposed household contacts**

not significantly higher in unvaccinated (38%, 95% CI 24–53) than fully vaccinated (25%, 18–33) household contacts (table 1). We estimated vaccine effectiveness at preventing infection (regardless of symptoms) with delta in the household setting to be 34% (bootstrap 95% CI −15 to 60). Sensitivity analyses using a 14 day threshold for time since second vaccination to study recruitment to denote fully vaccinated did not materially affect our estimates of vaccine effectiveness or SAR (data not shown). Although precision is restricted by the small sample size, this estimate is broadly consistent with vaccine effectiveness estimates for delta variant infection based on larger datasets.[9,16,17]

The vaccination status of 138 epidemiologically linked index cases of 204 delta variant-exposed household contacts was available (figure 1B, table 2). The SAR in household contacts exposed to fully vaccinated index cases was 25% (95% CI 15–35; 17 of 69), which is similar to the SAR in household contacts exposed to unvaccinated index cases (23% [15–31]; 23 of 100; table 2). The 53 PCR-positive contacts arose from household exposure to 39 PCR-positive index cases. Of these index cases who gave rise to secondary transmission, the proportion who were fully vaccinated (15 [38%] of 39) was similar to the proportion who were unvaccinated (16 [41%] of 39). The median number of days from the index cases' second vaccination to the day of recruitment for their respective contacts was 73 days (IQR 38–116). Time interval did not differ between index cases who transmitted infection to their contacts and those who did not (94 days [IQR 62–112] and 63 days [35–117], respectively; p=0·43).

18 of the 163 delta variant-infected index cases that led to contact enrolment were themselves recruited to ATACCC2 and serial URT samples were collected from them, allowing for more detailed virology and genome analyses. For 15 of these, their contacts were also recruited (13 household contacts and two non-household contacts). A corresponding PCR-positive household contact was identified for four of these 15 index cases (figure 1B). Genomic analysis showed that index–contact pairs were infected with the same delta variant sub-lineage in these instances, with one exception (figure 2A). In one household (number 4), an unvaccinated index case transmitted the delta variant to an unvaccinated contact, while another partially vaccinated contact was infected with a different delta sub-lineage (which was probably acquired outside the household). In the other three households (numbers 1–3), fully vaccinated index cases transmitted the delta variant to fully vaccinated household contacts, with high viral load in all cases, and temporal relationships between the viral load kinetics that were consistent with transmission from the index cases to their respective contacts (figure 2B).

Inclusion criteria for the modelling analysis selected 133 participant's viral load RNA trajectories from 163 PCR-positive participants (49 with the pre-alpha variant, 39 alpha, and 45 delta; appendix p 14). Of the 45 delta cases, 29 were fully vaccinated and 16 were unvaccinated; partially vaccinated cases were excluded. Of the 133 included cases, 29 (22%) were incident (ie, PCR negative at enrolment converting to PCR positive subsequently) and 104 (78%) were prevalent (ie, already PCR positive at enrolment). 15 of the prevalent cases had a clearly resolvable peak viral load. Figure 3 shows modelled viral RNA (ORF1ab) trajectories together with the viral RNA copy numbers measured for individual participants. The E-gene equivalent is shown in the appendix (p 2). Estimates derived from E-gene cycle threshold value data (appendix pp 5, 7, 9, 11) were similar to those for ORF1ab.

Although viral kinetics appear visually similar for all four groups of cases, we found quantitative differences in estimated viral growth rates and decline rates (tables 3, 4). Population (group-level) estimates of mean viral load decline rates based on ORF1ab cycle threshold value data varied in the range of 0·69–0·95 $\log_{10}$ units per mL per daxes 4; appendix p 10), indicating that a typical 10-day period was required for viral load to decline from peak to undetectable. A faster decline was seen in the alpha (pp=0·93), unvaccinated delta (pp=0·79), and fully vaccinated delta (pp=0·99) groups than in the pre-alpha group. The mean viral load decline rate of the fully vaccinated delta group was also faster than those of the alpha group (pp=0·84) and the unvaccinated delta group (pp=0·85). The differences in decline rates translate into a difference of about 3 days in the mean duration of the decline phase between the pre-alpha and delta vaccinated groups.

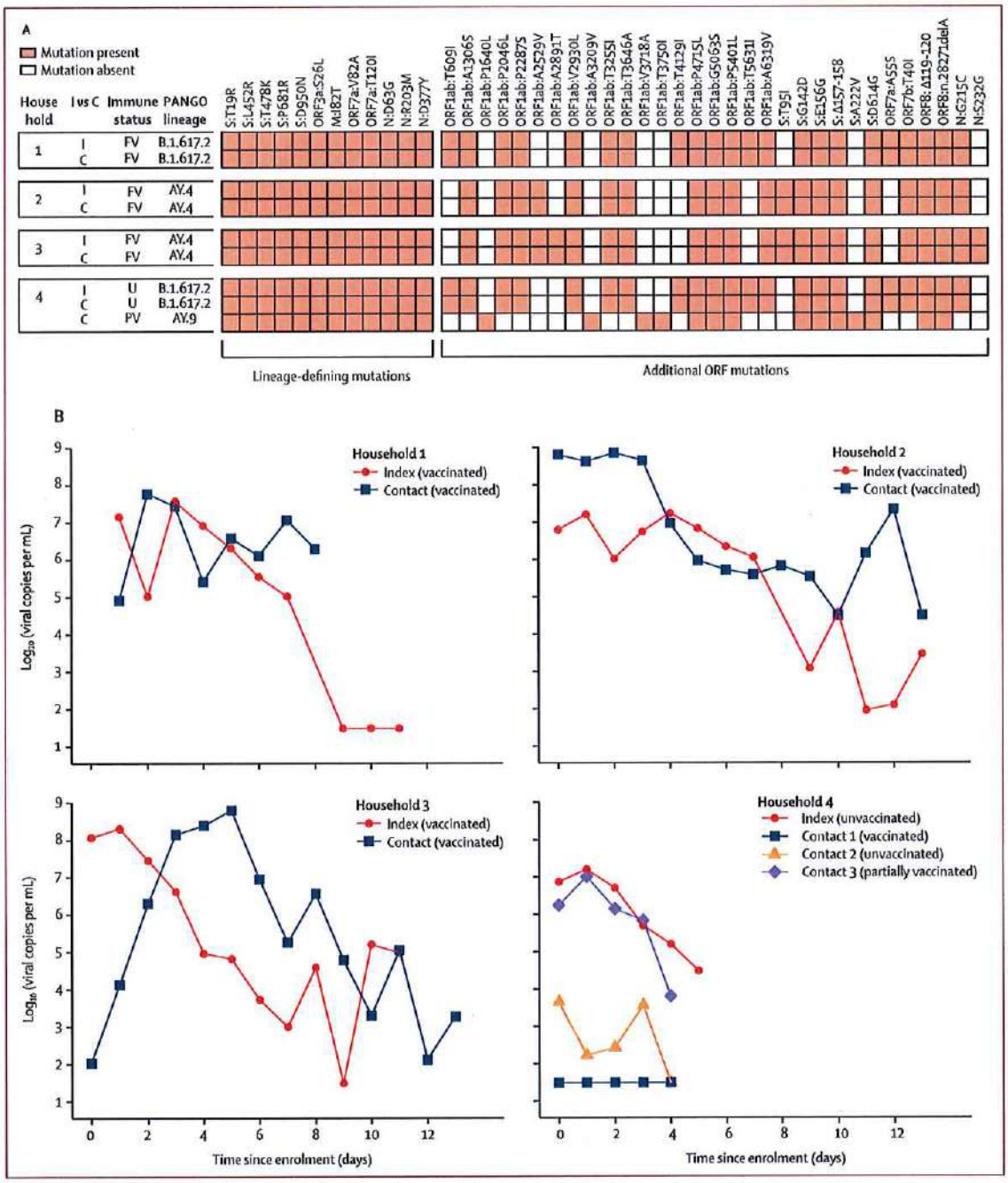
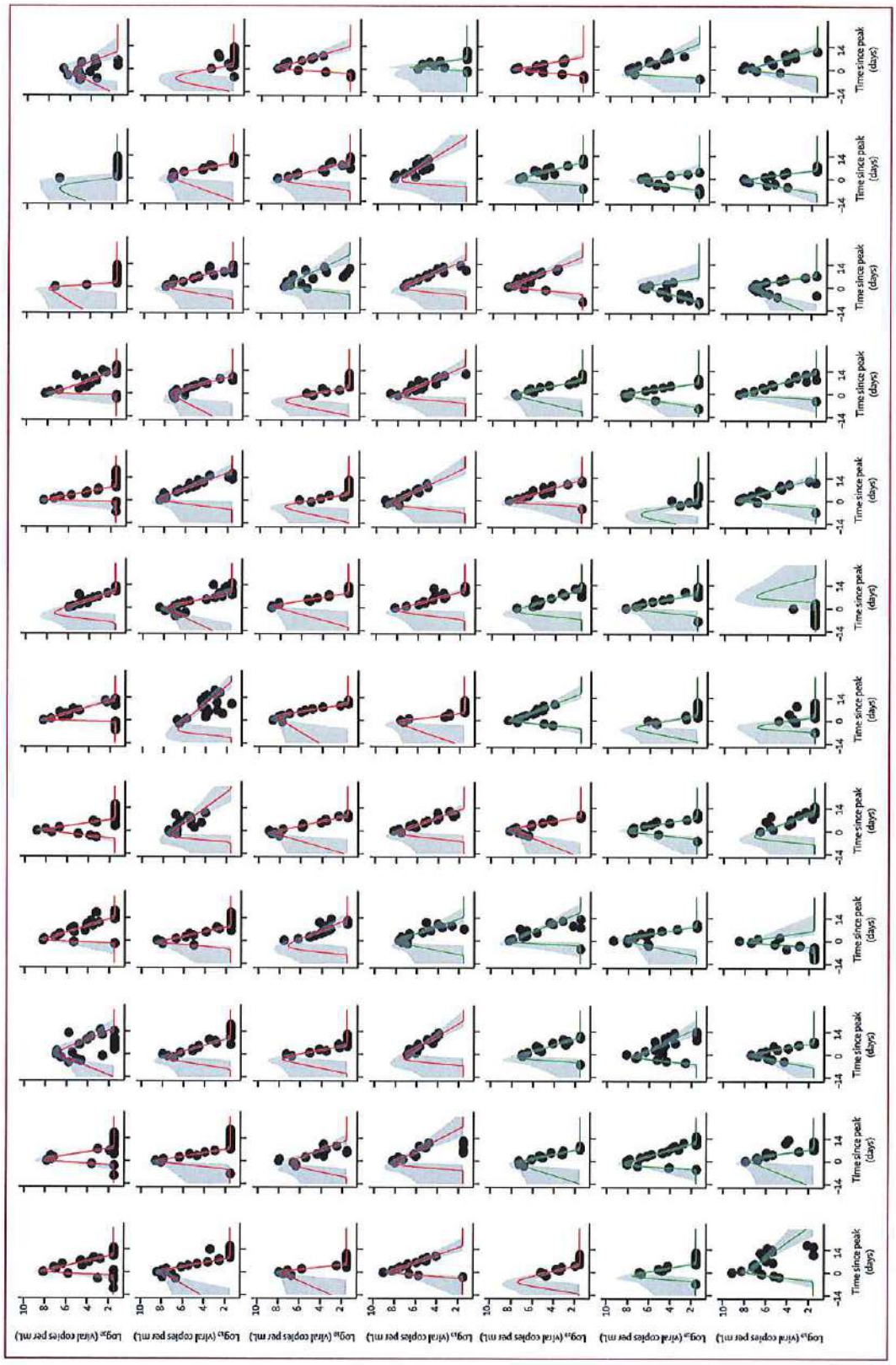


Figure 2: **Virological, epidemiological, and genomic evidence for transmission of the SARS-CoV-2 delta variant (B.1.617.2) in households**
(A) Genomic analysis of the four households with lineage-defining mutations for delta[18] and additional mutations within ORFs displayed to give insight into whether strains from individuals within the household are closely related. Lineages AY.4 and AY.9 are sub-lineages of delta. (B) Viral trajectories and vaccination status of the four index cases infected with the delta variant for whom infection was detected in their epidemiologically linked household contacts. All individuals had non-severe disease. Each plot shows an index case and their household contacts. Undetectable viral load measurements are plotted at the limit of detection ($10^{1\cdot43}$). C=contact. I=index case. FV=fully vaccinated. ORF=open reading frame. PV=partially vaccinated. U=unvaccinated.

Viral load growth rates were substantially faster than decline rates, varying in the range of 2·69–3·24 $\log_{10}$ units per mL per day between groups, indicating that a typical 3-day period was required for viral load to grow from undetectable to peak. Our power to infer differences in growth rates between groups was more restricted than for viral decline, but there was moderate evidence (pp=0·79) that growth rates were lower for



(Figure 3 continues on next page)

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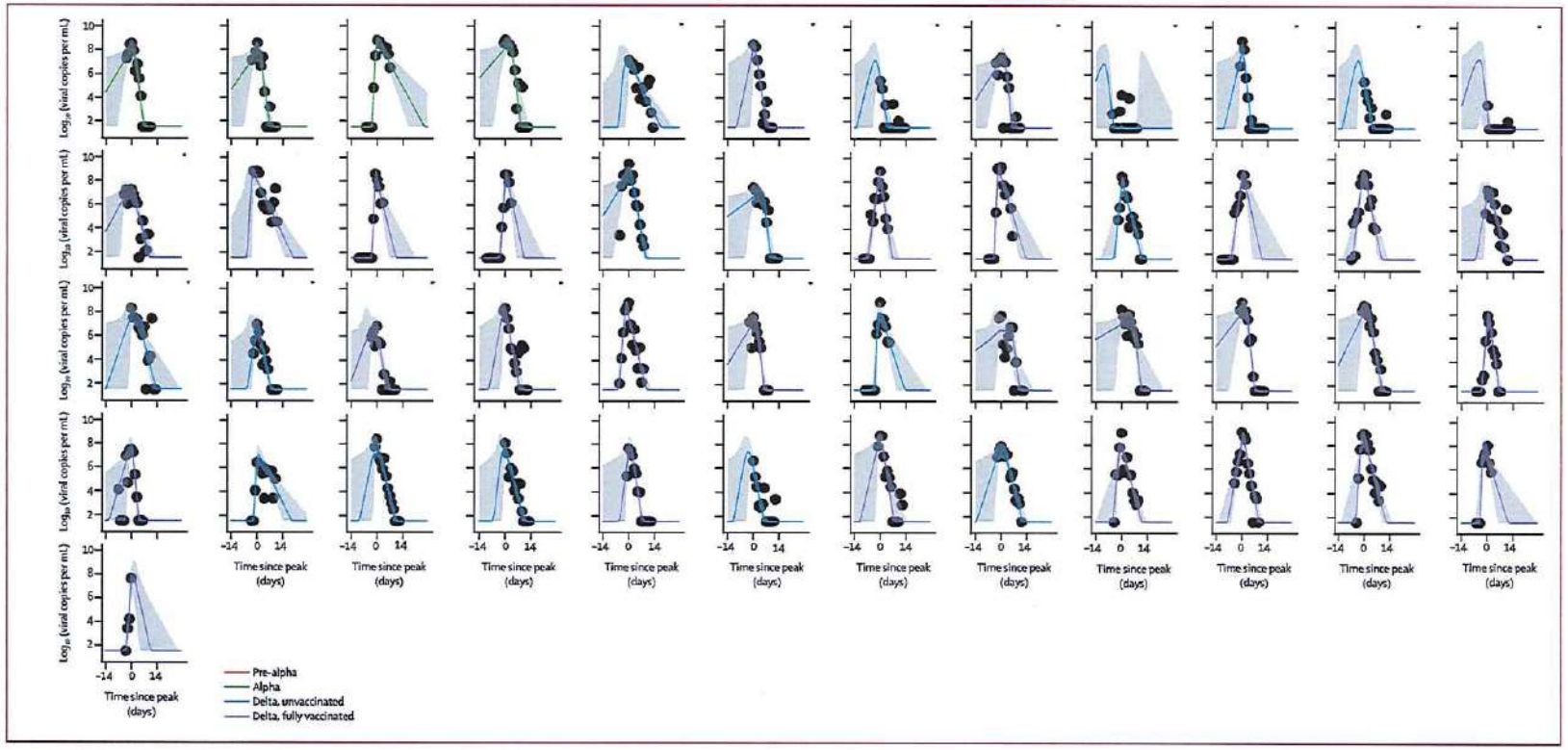

Figure 3: **ORF1ab viral load trajectories from 14 days before to 28 days after peak for 133 participants infected with pre-alpha or alpha variants (uncaccinated), or the delta variant (vaccinated and unvaccinated) variants**
Black circles are measured values, with the first datapoint for each participant being taken to the day of enrolment. Plots are rooted on the day of peak viral load for each participant, denoted as day 0 on the x-axis. Curves show the model posterior median estimate, with a 95% credible interval shading. 133 infected participants, comprising 114 contacts and 19 index cases. *Index cases.

	VL growth rate (95% CrI), log_{10} units per day	Posterior probability estimate is less than pre-alpha	Posterior probability estimate is less than alpha	Posterior probability estimate is less than delta (unvaccinated)	Posterior probability estimate is less than delta (fully vaccinated)
Pre-alpha (n=49)	3·24 (1·78–6·14)	..	0·44	0·27	0·21
Alpha (n=39)	3·13 (1·76–5·94)	0·56	..	0·32	0·25
Delta, unvaccinated (n=16)	2·81 (1·47–5·47)	0·73	0·68	..	0·44
Delta, fully vaccinated (n=29)	2·69 (1·51–5·17)	0·79	0·75	0·56	..

VL growth rates are shown as within-sample posterior mean estimates. Remaining columns show population (group-level) posterior probabilities that the estimate on that row is less than an estimate for a different group. Posterior probabilities are derived from 20 000 posterior samples and have sampling errors of <0·01. VL=viral load. CrI=credible interval.

Table 3: Estimates of VL growth rates for pre-alpha, alpha, and delta (unvaccinated and fully vaccinated) cases, derived from ORF1ab cycle threshold data

	VL decline rate (95% CrI), log_{10} units per day	Posterior probability estimate is larger than pre-alpha	Posterior probability estimate is larger than alpha	Posterior probability estimate is larger than delta (unvaccinated)	Posterior probability estimate is larger than delta (fully vaccinated)
Pre-alpha (n=49)	0·69 (0·58–0·81)	..	0·07	0·21	0·01
Alpha (n=39)	0·82 (0·67–1·01)	0·93	..	0·60	0·16
Delta, unvaccinated (n=16)	0·79 (0·59–1·04)	0·79	0·40	..	0·15
Delta, fully vaccinated (n=29)	0·95 (0·76–1·18)	0·99	0·84	0·85	..

VL decline rates are shown as within-sample posterior mean estimates. Remaining columns show population (group-level) posterior probabilities that the estimate on that row is less than an estimate for a different group. Posterior probabilities are derived from 20 000 posterior samples and have sampling errors of <0·01. VL=viral load. CrI=credible interval.

Table 4: Estimates of VL decline rates for pre-alpha, alpha, and delta (unvaccinated and fully vaccinated) cases, derived from ORF1ab cycle threshold data

those in the vaccinated delta group than in the pre-alpha group.

We estimated mean peak viral load for 50-year-old adults to be 8·14 (95% CrI 7·95 to 8·32) log_{10} copies per mL, but peak viral load did not differ by variant or vaccination status. However, we estimated that peak viral load increases with age (pp=0·96 that the slope of peak viral load with log[age] was >0), with an estimated slope of 0·24 (95% CrI –0·02 to 0·49) log_{10} copies per mL per unit change in log(age). This estimate translates to a difference of 0·39 (–0·03 to 0·79) in mean peak log_{10} copies per mL between those aged 10 years and 50 years.

Within-group individual participant estimates of viral load growth rate were positively correlated with peak viral load, with a correlation coefficient estimate of 0·42 (95% CrI 0·13 to 0·65; appendix p 8). Hence, individuals with faster viral load growth tend to have higher peak viral load. The decline rate of viral load was also negatively correlated with viral load growth rate, with a correlation coefficient estimate of –0·44 (95% CrI –0·67 to –0·18), illustrating that individuals with faster viral load growth tend to experience slower viral load decline.

Discussion

Households are the site of most SARS-CoV-2 transmission globally.[19] In our cohort of densely sampled household contacts exposed to the delta variant, SAR was 38% in unvaccinated contacts and 25% in fully vaccinated contacts. This finding is consistent with the known protective effect of COVID-19 vaccination against infection.[8,9] Notwithstanding, these findings indicate continued risk of infection in household contacts despite vaccination. Our estimate of SAR is higher than that reported in fully vaccinated household contacts exposed before the emergence of the delta variant.[1,20,21] The time interval between vaccination and study recruitment was significantly higher in fully vaccinated PCR-positive contacts than fully vaccinated PCR-negative contacts, suggesting that susceptibility to infection increases with time as soon as 2–3 months after vaccination—consistent with waning protective immunity. This potentially important observation is consistent with recent large-scale data and requires further investigation.[17] Household SAR for delta infection, regardless of vaccination status, was 26% (95% CI 20–32), which is higher than estimates of UK national surveillance data (10·8% [10·7–10·9]).[10] However, we sampled contacts daily, regardless of symptomatology, to actively identify infection with high sensitivity. By contrast, symptom-based, single-timepoint surveillance testing probably underestimates the true SAR, and potentially also overestimates vaccine effectiveness against infection.

We identified similar SAR (25%) in household contacts exposed to fully vaccinated index cases as in those exposed to unvaccinated index cases (23%). This finding indicates that breakthrough infections in fully vaccinated people can efficiently transmit infection in the household setting. We identified 12 household transmission events between fully vaccinated index case–contact pairs; for three of these, genomic sequencing confirmed that the index case and

contact were infected by the same delta variant sub-lineage, thus substantiating epidemiological data and temporal relationships of viral load kinetics to provide definitive evidence for secondary transmission. To our knowledge, one other study has reported that transmission of the delta variant between fully vaccinated people was a point-source nosocomial outbreak—a single health-care worker with a particular delta variant sub-lineage in Vietnam.[22]

Daily longitudinal sampling of cases from early (median 4 days) after exposure for up to 20 days allowed us to generate high-resolution trajectories of URT viral load over the course of infection. To date, two studies have sequentially sampled community cases of mild SARS-CoV-2 infection, and these were from highly specific population groups identified through asymptomatic screening programmes (eg, for university staff and students[23] and for professional athletes[24]).

Our most predictive model of viral load kinetics estimated mean peak $\log_{10}$ viral load per mL of 8·14 (95% CrI 7·95–8·32) for adults aged 50 years, which is very similar to the estimate from a 2021 study using routine surveillance data.[25] We found no evidence of variation in peak viral load by variant or vaccination status, but we report some evidence of modest but significant (pp=0·95) increases in peak viral load with age. Previous studies of viral load in children and adults[4,25,26] have not used such dense sequential sampling of viral load and have, therefore, been restricted in their power to resolve age-related differences; the largest such study[25] reported a similar difference between children and adults to the one we estimated. We found the rate of viral load decline was faster for vaccinated individuals with delta infection than all other groups, and was faster for individuals in the alpha and unvaccinated delta groups than those with pre-alpha infection.

For all variant vaccination groups, the variation between participants seen in viral load kinetic parameter estimates was substantially larger than the variation in mean parameters estimated between groups. The modest scale of differences in viral kinetics between fully vaccinated and unvaccinated individuals with delta infection might explain the relatively high rates of transmission seen from vaccinated delta index cases in our study. We found no evidence of lower SARs from fully vaccinated delta index cases than from unvaccinated ones. However, given that index cases were identified through routine symptomatic surveillance, there might have been a selection bias towards identifying untypically symptomatic vaccine breakthrough index cases.

The differences in viral kinetics we found between the pre-alpha, alpha, and delta variant groups suggest some incremental, but potentially adaptive, changes in viral dynamics associated with the evolution of SARS-CoV-2 towards more rapid viral clearance. Our study provides the first evidence that, within each variant or vaccination group, viral growth rate is positively correlated with peak viral load, but is negatively correlated with viral decline rate. This finding suggests that individual infections during which viral replication is initially fastest generate the highest peak viral load and see the slowest viral clearance, with the latter not just being due to the higher peak. Mechanistically, these data suggest that the host and viral factors determining the initial growth rate of SARS-CoV-2 have a fundamental effect on the trajectory throughout infection, with faster replication being more difficult (in terms of both peak viral load and the subsequent decline of viral load) for the immune response to control. Analysis of sequentially sampled immune markers during infection might give insight into the immune correlates of these early differences in infection kinetics. It is also possible that individuals with the fastest viral load growth and highest peaks contribute disproportionately to community transmission, a hypothesis that should be tested in future studies.

Several population-level, single-timepoint sampling studies using routinely available data have found no major differences in cycle threshold values between vaccinated and unvaccinated individuals with delta variant infection.[10,27,28] However, as the timepoint of sampling in the viral trajectory is unknown, this restricts the interpretation of such results. Two other studies longitudinally sampled vaccinated and unvaccinated individuals with delta variant infection.[23,29] A retrospective cohort of hospitalised patients in Singapore[29] also described a faster rate of viral decline in vaccinated versus unvaccinated individuals with delta variant, reporting somewhat larger differences in decline rates than we estimated here. However, this disparity might be accounted for by the higher severity of illness in unvaccinated individuals in the Singaporean study (almost two-thirds having pneumonia, one-third requiring COVID-19 treatment, and a fifth needing oxygen) than in our study, given that longer viral shedding has been reported in patients with more severe illness.[30] A longitudinal sampling study in the USA reported that pre-alpha, alpha, and delta variant infections had similar viral trajectories.[24] The study also compared trajectories in vaccinated and unvaccinated individuals, reporting similar proliferation phases and peak cycle threshold values, but more rapid clearance of virus in vaccinated individuals. However, this study in the USA stratified by vaccination status and variant separately, rather than jointly, meaning vaccinated individuals with delta infection were being compared with, predominantly, unvaccinated individuals with pre-alpha and alpha infection. Moreover, sampling was done as part of a professional sports player occupational health screening programme, making the results not necessarily representative of typical community infections.

Our study has limitations. First, we recruited only contacts of symptomatic index cases as our study recruitment is derived from routine contact-tracing notifications. Second, index cases were defined as the first household member to have a PCR-positive swab, but we cannot exclude the possibility that another household member might already have been infected and transmitted

to the index case. Third, recording of viral load trajectories is subject to left censoring, where the growth phase in prevalent contacts (already PCR-positive at enrolment) was missed for a proportion of participants. However, we captured 29 incident cases and 15 additional cases on the upslope of the viral trajectory, providing valuable, informative data on viral growth rates and peak viral load in a subset of participants. Fourth, owing to the age-stratified rollout of the UK vaccination programme, the age of the unvaccinated, delta variant-infected participants was lower than that of vaccinated participants. Thus, age might be a confounding factor in our results and, as discussed, peak viral load was associated with age. However, it is unlikely that the higher SAR observed in the unvaccinated contacts would have been driven by younger age rather than the absence of vaccination and, to our knowledge, there is no published evidence showing increased susceptibility to SARS-CoV-2 infection with decreasing age.[31] Finally, although we did not perform viral culture here—which is a better proxy for infectiousness than RT-PCR—two other studies[27,32] have shown cultivable virus from around two-thirds of vaccinated individuals infected with the delta variant, consistent with our conclusions that vaccinated individuals still have the potential to infect others, particularly early after infection when viral loads are high and most transmission is thought to occur.[30]

Our findings help to explain how and why the delta variant is being transmitted so effectively in populations with high vaccine coverage. Although current vaccines remain effective at preventing severe disease and deaths from COVID-19, our findings suggest that vaccination alone is not sufficient to prevent all transmission of the delta variant in the household setting, where exposure is close and prolonged. Increasing population immunity via booster programmes and vaccination of teenagers will help to increase the currently limited effect of vaccination on transmission, but our analysis suggests that direct protection of individuals at risk of severe outcomes, via vaccination and non-pharmacological interventions, will remain central to containing the burden of disease caused by the delta variant.

Contributors

AS, JD, MZ, NMF, WB, and ALal conceptualised the study. AS, SH, JD, KJM, AK, JLB, MGW, ND-F, RV, RK, JF, CT, AVK, JC, VQ, EC, JSN, SH, EM, TP, HH, CL, JS, SB, JP, CA, SA, and NMF were responsible for data curation and investigation. AS, SH, KJM, JLB, AC, NMF, and ALal did the formal data analysis. MAC, AB, DJ, SM, JE, PSF, SD, and ALac did the laboratory work. RV, RK, JF, CT, AVK, JC, VQ, EC, JSN, SH, EM, and SE oversaw the project. AS, SH, JD, KJM, JLB, NMF, and ALal accessed and verified the data. JD, MZ, and ALal acquired funding. NMF sourced and oversaw the software. AS and ALal wrote the initial draft of the manuscript. AS, JD, GPT, MZ, NMF, SH, and ALal reviewed and edited the manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Declaration of interests

NMF reports grants from UK Medical Research Council, UK National Institute of Health Research, UK Research and Innovation, Community Jameel, Janssen Pharmaceuticals, the Bill & Melinda Gates Foundation, and Gavi, the Vaccine Alliance; consulting fees from the World Bank; payment or honoraria from the Wellcome Trust; travel expenses from WHO; advisory board participation for Takeda; and is a senior editor of the *eLife* journal. All other authors declare no competing interests.

Data sharing

An anonymised, de-identified version of the dataset can be made available upon request to allow all results to be reproduced. Modelling code will also be made publicly available on the GitHub repository.

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Reduced Risk of Reinfection with SARS-CoV-2 After COVID-19 Vaccination — Kentucky, May–June 2021

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On August 6, 2021, this report was posted as an MMWR *Early Release on the* MMWR *website (https://www.cdc.gov/mmwr).*

Although laboratory evidence suggests that antibody responses following COVID-19 vaccination provide better neutralization of some circulating variants than does natural infection (*1*,*2*), few real-world epidemiologic studies exist to support the benefit of vaccination for previously infected persons. This report details the findings of a case-control evaluation of the association between vaccination and SARS-CoV-2 reinfection in Kentucky during May–June 2021 among persons previously infected with SARS-CoV-2 in 2020. Kentucky residents who were not vaccinated had 2.34 times the odds of reinfection compared with those who were fully vaccinated (odds ratio [OR] = 2.34; 95% confidence interval [CI] = 1.58–3.47). These findings suggest that among persons with previous SARS-CoV-2 infection, full vaccination provides additional protection against reinfection. To reduce their risk of infection, all eligible persons should be offered vaccination, even if they have been previously infected with SARS-CoV-2.*

Kentucky residents aged ≥18 years with SARS-CoV-2 infection confirmed by positive nucleic acid amplification test (NAAT) or antigen test results† reported in Kentucky's National Electronic Disease Surveillance System (NEDSS) during March–December 2020 were eligible for inclusion. NEDSS data for all Kentucky COVID-19 cases were imported into a REDCap database that contains laboratory test results and case investigation data, including dates of death for deceased patients reported to public health authorities (*3*). The REDCap database was queried to identify previously infected persons, excluding COVID-19 cases resulting in death before May 1, 2021. A case-patient was defined as a Kentucky resident with laboratory-confirmed SARS-CoV-2 infection in 2020 and a subsequent positive NAAT or antigen test result during May 1–June 30, 2021. May and June were selected because of vaccine supply and eligibility requirement considerations; this period was more likely to reflect resident choice to be vaccinated, rather than eligibility to receive vaccine.§ Control participants were Kentucky residents with laboratory-confirmed SARS-CoV-2 infection in 2020 who were not reinfected through June 30, 2021. Case-patients and controls were matched on a 1:2 ratio based on sex, age (within 3 years), and date of initial positive SARS-CoV-2 test (within 1 week). Date of initial positive test result refers to the specimen collection date, if available. The report date in NEDSS was used if specimen collection date was missing. Random matching was performed to select controls when multiple possible controls were available to match per case (*4*).

Vaccination status was determined using data from the Kentucky Immunization Registry (KYIR). Case-patients and controls were matched to the KYIR database using first name, last name, and date of birth. Case-patients were considered fully vaccinated if a single dose of Janssen (Johnson & Johnson) or a second dose of an mRNA vaccine (Pfizer-BioNTech or Moderna) was received ≥14 days before the reinfection date. For controls, the same definition was applied, using the reinfection date of the matched case-patient. Partial vaccination was defined as receipt of ≥1 dose of vaccine, but either the vaccination series was not completed or the final dose was received <14 days before the case-patient's reinfection date. Using conditional logistic regression, ORs and CIs were used to compare no vaccination and partial vaccination with full vaccination among case-patients and controls. SAS (version 9.4; SAS Institute) was used for matching and statistical analyses. This activity was reviewed by CDC and was conducted consistent with applicable federal law and CDC policy.¶

Overall, 246 case-patients met eligibility requirements and were successfully matched by age, sex, and date of initial infection with 492 controls. Among the population included in the analysis, 60.6% were female, and 204 (82.9%) case-patients were initially infected during October–December 2020

* https://www.cdc.gov/vaccines/covid-19/clinical-considerations/covid-19-vaccines-us.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fvaccines%2Fcovid-19%2Finfo-by-product%2Fclinical-considerations.html#CoV-19-vaccination

† https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing-overview.html

§ May and June were selected for two primary reasons. First, when vaccination supplies were low, some previously infected persons were deferring vaccination for 90 days to allow never-infected persons priority for available vaccine; however, by May 2021, deferral for 90 days was no longer a reason for those infected in 2020 to remain unvaccinated. Second, although vaccination eligibility was initially restricted based on age, comorbidities, and occupation, by April 5, 2021, all Kentucky residents aged ≥16 years became eligible for vaccination (https://chfs.ky.gov/agencies/dph/covid19/Cv19VaccineFAskedQ.pdf). Thus, vaccination status in May or June 2021 might more accurately reflect choice rather than eligibility to be vaccinated.

¶ 45 C.F.R. part 46.102(l)(2), 21 C.F.R. part 56; 42 U.S.C. Sect. 241(d); 5 U.S.C. Sect. 552a; 44 U.S.C. Sect. 3501 et seq.

Summary

What is already known about this topic?

Reinfection with human coronaviruses, including SARS-CoV-2, the virus that causes COVID-19, has been documented. Currently, limited evidence concerning the protection afforded by vaccination against reinfection with SARS-CoV-2 is available.

What is added by this report?

Among Kentucky residents infected with SARS-CoV-2 in 2020, vaccination status of those reinfected during May–June 2021 was compared with that of residents who were not reinfected. In this case-control study, being unvaccinated was associated with 2.34 times the odds of reinfection compared with being fully vaccinated.

What are the implications for public health practice?

To reduce their likelihood for future infection, all eligible persons should be offered COVID-19 vaccine, even those with previous SARS-CoV-2 infection.

(Table 1). Among case-patients, 20.3% were fully vaccinated compared with 34.3% of controls (Table 2). Kentucky residents with previous infections who were unvaccinated had 2.34 times the odds of reinfection (OR = 2.34; 95% CI = 1.58–3.47) compared with those who were fully vaccinated; partial vaccination was not significantly associated with reinfection (OR = 1.56; 95% CI = 0.81–3.01).

Discussion

This study found that among Kentucky residents who were previously infected with SARS-CoV-2 in 2020, those who were unvaccinated against COVID-19 had significantly higher likelihood of reinfection during May and June 2021. This finding supports the CDC recommendation that all eligible persons be offered COVID-19 vaccination, regardless of previous SARS-CoV-2 infection status.

Reinfection with SARS-CoV-2 has been documented, but the scientific understanding of natural infection-derived immunity is still emerging (*5*). The duration of immunity resulting from natural infection, although not well understood, is suspected to persist for ≥90 days in most persons.** The emergence of new variants might affect the duration of infection-acquired immunity, and laboratory studies have shown that sera from previously infected persons might offer weak or inconsistent responses against several variants of concern (*2,6*). For example, a recent laboratory study found that sera collected from previously infected persons before they were vaccinated provided a relatively weaker, and in some cases absent, neutralization response to the B.1.351 (Beta) variant when compared with the original Wuhan-Hu-1 strain (*1*). Sera from the same persons after vaccination showed a heightened neutralization response to the Beta variant, suggesting that vaccination enhances the immune response even to a variant to which the infected person had not been previously exposed. Although such laboratory evidence continues to suggest that vaccination provides improved neutralization of SARS-CoV-2 variants, limited evidence in real-world settings to date corroborates the findings that vaccination can provide improved protection for previously infected persons. The findings from this study suggest that among previously infected persons, full vaccination is associated with reduced likelihood of reinfection, and, conversely, being unvaccinated is associated with higher likelihood of being reinfected.

The lack of a significant association with partial versus full vaccination should be interpreted with caution given the small numbers of partially vaccinated persons included in the analysis (6.9% of case-patients and 7.9% of controls), which limited statistical power. The lower odds of reinfection among the partially vaccinated group compared with the unvaccinated group is suggestive of a protective effect and consistent with findings from previous studies indicating higher titers after the first mRNA vaccine dose in persons who were previously infected (*7,8*).

The findings in this report are subject to at least five limitations. First, reinfection was not confirmed through whole genome sequencing, which would be necessary to definitively prove that the reinfection was caused from a distinct virus relative to the first infection. Although in some cases the repeat positive test could be indicative of prolonged viral shedding or failure to clear the initial viral infection (*9*), given the time between initial and subsequent positive molecular tests among participants in this study, reinfection is the most likely explanation. Second, persons who have been vaccinated are possibly less likely to get tested. Therefore, the association of reinfection and lack of vaccination might be overestimated. Third, vaccine doses administered at federal or out-of-state sites are not typically entered in KYIR, so vaccination data are possibly missing for some persons in these analyses. In addition, inconsistencies in name and date of birth between KYIR and NEDSS might limit ability to match the two databases. Because case investigations include questions regarding vaccination, and KYIR might be updated during the case investigation process, vaccination data might be more likely to be missing for controls. Thus, the OR might be even more favorable for vaccination. Fourth, although case-patients and controls were matched based on age, sex, and date of initial infection, other unknown confounders might be present. Finally, this is a retrospective study design using data from a single state during a 2-month period; therefore, these findings cannot be used to infer causation. Additional prospective studies with larger populations are warranted to support these findings.

** https://www.cdc.gov/coronavirus/2019-ncov/hcp/duration-isolation.html

TABLE 1. Demographic characteristics of COVID-19 patients with reinfection (case-patients) and COVID-19 patients who were not reinfected (control participants) — Kentucky, May–June 2021

Characteristic	No. (%) Case-patients* (n = 246)	Control participants† (n = 492)
Age group, yrs		
18–29	46 (18.7)	89 (18.1)
30–39	37 (15.0)	83 (16.9)
40–49	43 (17.5)	80 (16.3)
50–59	44 (17.9)	88 (17.9)
60–69	27 (11.0)	51 (10.4)
70–79	28 (11.4)	58 (11.8)
≥80	21 (8.5)	43 (8.7)
Sex		
Female	149 (60.6)	298 (60.6)
Month of initial infection in 2020		
March	0 (0)	3 (0.6)
April	7 (2.8)	11 (2.2)
May	2 (0.8)	2 (0.4)
June	4 (1.6)	11 (2.2)
July	8 (3.3)	17 (3.5)
August	8 (3.3)	13 (2.6)
September	13 (5.3)	22 (4.5)
October	36 (14.6)	78 (15.9)
November	72 (29.3)	141 (28.7)
December	96 (39.0)	194 (39.4)

* Case-patients were eligible for inclusion if initial infection occurred during March–December 2020, and a subsequent positive nucleic acid amplification or antigen test result was received during May–June 2021 (using date of specimen collection). Cases for analyses were restricted to persons aged ≥18 years at time of reinfection.

† Controls were matched by sex, age (within 3 years), and time of initial infection diagnosis (within 7 days).

These findings suggest that among persons with previous SARS-CoV-2 infection, full vaccination provides additional protection against reinfection. Among previously infected Kentucky residents, those who were not vaccinated were more than twice as likely to be reinfected compared with those with full vaccination. All eligible persons should be offered vaccination, including those with previous SARS-CoV-2 infection, to reduce their risk for future infection.

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TABLE 2. Association of SARS-CoV-2 reinfection* with COVID-19 vaccination status — Kentucky, May–June 2021

Vaccination status	No. (%) Case-patients	Control participants	OR (95% CI)†
Not vaccinated	179 (72.8)	284 (57.7)	2.34 (1.58–3.47)
Partially vaccinated¶	17 (6.9)	39 (7.9)	1.56 (0.81–3.01)
Fully vaccinated§	50 (20.3)	169 (34.3)	Ref
Total	246 (100)	492 (100)	—

Abbreviations: CI = confidence interval; NAAT = nucleic acid amplification test; OR = odds ratio; Ref = referent group.

* All case-patients (reinfected) and control participants (not reinfected) had previous SARS-CoV-2 infection documented by positive NAAT or antigen test results during March–December 2020. Reinfection was defined as receipt of positive NAAT or antigen test results during May 1–June 30, 2021.

† Estimated based on conditional logistic regression.

§ Case-patients were considered partially vaccinated if ≥1 dose of vaccine was received, but the vaccination series was either not completed or the final dose was received <14 days before their reinfection date. For control participants, the same criteria were applied, using the matched case-patient's reinfection date.

¶ Case-patients and control participants were considered fully vaccinated if a complete COVID-19 vaccine series was received ≥14 days before the case-patient's reinfection date.

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Vacunación

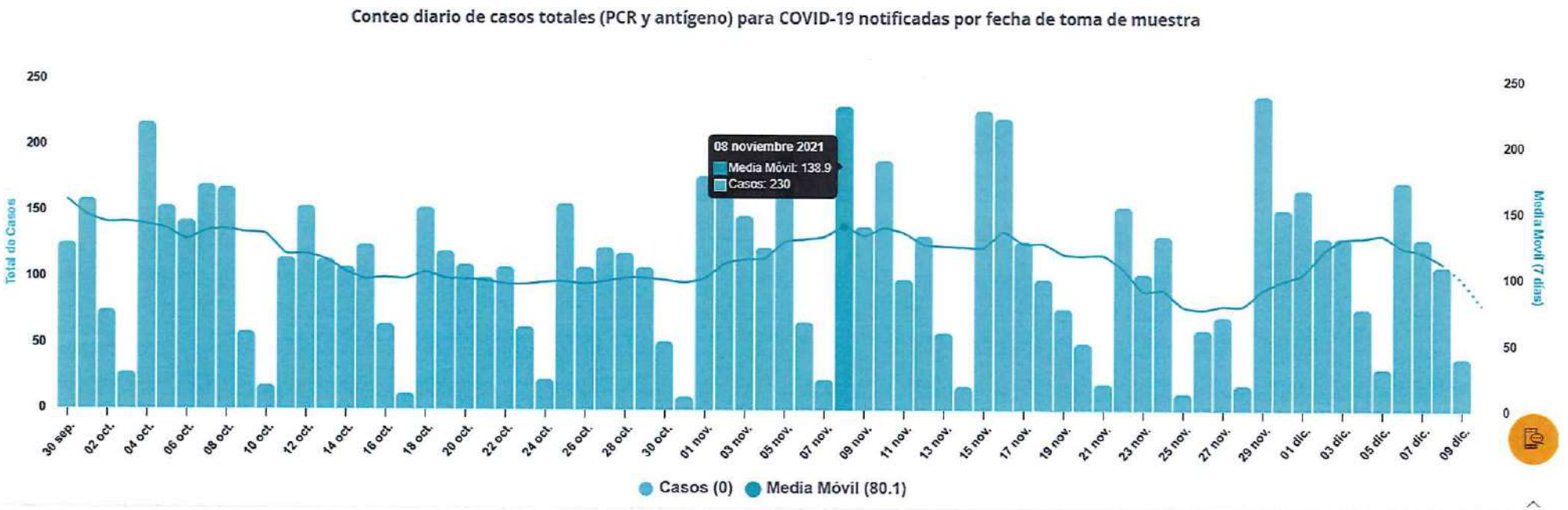
Actualizado el 12/12/2021

Datos del proceso de vacunación contra el COVID-19

2,698,689	2,408,552	87.7%	78.3%
PERSONAS APTAS (5 AÑOS O MÁS) CON AL MENOS UNA DOSIS	PERSONAS APTAS (5 AÑOS O MÁS) CON SERIE DE DOSIS COMPLETADA	PORCIENTO DE PERSONAS APTAS (5 AÑOS O MÁS) CON AL MENOS UNA DOSIS	PORCIENTO DE PERSONAS APTAS (5 AÑOS O MÁS) CON SERIE DE DOSIS COMPLETADA
Datos recogidos al 2021-12-11 *Definicion de Medida	Datos recogidos al 2021-12-11 *Definicion de Medida	Datos recogidos al 2021-12-11 *Definicion de Medida	Datos recogidos al 2021-12-11 *Definicion de Medida

Distribución de la población por sexo y grupo de edad

Puerto Rico Health Department, COVID-19 en Cifras en Puerto Rico, *Vacunación*, https://covid19datos.salud.gov.pr/#vacunacion



Puerto Rico Health Department, COVID-19 en Cifras en Puerto Rico, *Casos*, https://covid19datos.salud.gov.pr/#casos

Municipio Autónomo de San Juan

[signature]
Facilidad de Salud

Tel. (787) 480-5275

Apellido Paterno	Apellido Materno	Nombre	Edad:
Otero	Santiago	Ofelia	48

Dirección:

Fecha: 23 / IX / 2021
día mes año

Diagnóstico: Exoneración 2da dosis de Covid 19 Pfizer

Pte 48 a/o fem hx Covid 19 el oct. 22 del 2020 con un amigo, se hizo prueba el 5 nov. 2020 (Molecular +) fue obligada a vacunarse el 21 de agosto 2021, sin necesidad. Se exonera de 2da dosis.

☐ Repetir ______ ☐ No Repetir

Dra. Sylvia Muñoz Fidalgo [signature] MD
Nombre del Médico en letra de molde — Firma del Médico

Lic 14729 — DEA ______

NPI 1366487892 — DM ______

Dirección ______

Curriculum Vitae

Iris R. Cardona, M.D.

Mansiones de Villanova BD1-18
San Juan, Puerto Rico
Phone 787-690-0709
rmeaux@hotmail.com

Post Graduate Education

Fellowship in Pediatric Infectious Diseases
University Pediatric Hospital, University of Puerto Rico
July 1990-June 1992

Residency in Pediatrics. University Pediatric Hospital,
University of Puerto Rico. July 1987-June 1990.

Internship in Pediatrics. University Pediatric Hospital,
University of Puerto Rico, July 1987- June 1988.

Other Education and Degrees

Doctor in Medicine, University of Puerto Rico
School of Medicine, May 1987.

Bachelor of Science in Biology. University of Puerto Rico,
Magna Cum Laude, May 1983.

Licenses and Boards

Puerto Rico State Board License since 1990.
American Board of Pediatrics, No. 061174, 1997
National Boards Certification 1990.
FLEX 1989.

Postgraduate/Scientific Courses

Epidemiology Prevention and Control of Vaccine Preventable Diseases,
Centers for Disease Control and Prevention, 1994

Medical Immunology, University of Puerto Rico, Graduate School of Microbiology, 1990.

Medical Mycology, University of Puerto Rico, Graduate School of Microbiology, 1991.

Work Experience

Puerto Rico Health Department, Chief Medical Officer, March 2021-present

Puerto Rico Health Department, COVID-19 Vaccination Program IC, December 2020-present

Puerto Rico Health Department, Undersecretary of Health
June 2020-December 2020

UPR School of Medicine, Department of Pediatrics, Associate Professor Pediatrics 2016-present.

San Jorge Children Hospital, President Infection Control Committee, February 2015 to December 2019

Immunization Program, Puerto Rico Department of Health, Clinical Advisor and Adolescent Immunization Coordinator, October 2014 to present

Private Practice at Centro Pediátrico Country Club since September 2014-present

Bristol Myers Squibb, Puerto Rico, Medical Affairs, Virology Division May 2013- August 2014.

Immunization Program, Puerto Rico Department of Health, Clinical Advisor 2000 - 2013

Private Practice at Centro Pediatrico Country Club 1992 to 2013

Pediatric AIDS Program at Puerto Rico Department of
Health, Clinical Services Coordinator, 1992-1994.

San Pablo Hospital, Department of Pediatrics, Infectious Diseases Medical Consultant, 1992-1995.

PR Department of Health, Strategic Planning Committee, Member 2014-present

UPR Carolina Hospital, Department of Pediatrics, Faculty Member, 1991-1993.

UPR School of Medicine, Department of Pediatrics, Faculty 1991-993

Member of Speaker Bureau:
GSK 2002-2013
MSD 2008-2013
Medimmune 2008- 2013
Novartis 2011-2013
University of Puerto Rico School of Public Health, Invited Professor on the topic of Vaccine Preventable Diseases
University of Puerto Rico, School of Pharmacy, Post Graduate Education Office, Medical Sciences Campus.
Advisory Board GSK, 2010
Advisory Board MSD, 2009

Affiliations

Puerto Rico Pediatric Society, Board Member, 2018 to present
Puerto Rico College of Physicians
Puerto Rico Health Council, member since 2011
University of Puerto Rico, Pediatric Alumni Society

Hospital Affiliations

San Jorge Children's Hospital

Honors

Alpha Omega Alpha, Honor Medical Society membership 2021
PR Infectious Diseases Society Award, April 2021
Doctor's Choice Awards 2013, 2014, 2015, 2016, 2017, 2018, 2019,2021
Immunization Champion Award at Immunization Conference IMCO, 2017

Presentations

Immunization Update, Puerto Rico Pediatric Society Annual Convention, 2019
Restoring Immunization Services after Major Disasters in Puerto Rico, presented at Puerto Rico Pediatric Society Symposium, 2018
Immunization and, Autism Controversy 2016, Puerto Rico Pediatric Society Annual Convention, 2016
National Immunization Survey, Puerto Rico 2014, Presented at Puerto Rico Health Department 2015, 2016, 2017
The Vaccine Controversy, Myths and Realities at PR Second Immunization Congress 2014
Adolescent Immunization, presented at Puerto Rico Pediatric Society Annual Convention, February 2012.
Adult Immunization, Puerto Rico Pharmacist Convention, 2011, 2012.
Immunization of Preemies: Special Circumstances, Puerto Rico Pediatric Society Neonatal Symposium, February 2011
Cervical Cancer and Beyond, at General Medicine Congress, San Juan, PR, February 2011.
Cervical Cancer and HPV Immunization, Presented at Forum for Cervical Cancer Prevention at Puerto Rico Comprehensive Cancer Center, University of Puerto Rico, January 2011.
Immunization: Special Challenges in Puerto Rico, Presented at Puerto Rico Pediatric Society Annual Convention, 2010.
Immunization Update presented at AMPRE Annual Convention 2010.
Epidemiology of Vaccine Preventable Diseases, Lecture at Graduate School of Public Health, University of Puerto Rico School of Medicine: 2006, 2007, 2008, 2009, 2010, 20111,2012,2013,2014. 2015, 2016
Adolescent Immunization, presented at Forum for Adolescent Immunization, Puerto Rico Department of Health, 2010.
Neurobiological Effects of Vaccines, Presented at Neuroscience and Epilepsy Conference, San Juan Puerto Rico, 2010
Cervical Cancer Prevention, Presented at LULAC Women Convention, San Juan P.R, 2010.
Dengue Medical Management, University of Puerto Rico School of Pharmacy, 2010.
Immunization Mandates, presented at United States Black and Hispanic Caucus of State Legislators 2009 Annual Convention, 2009.
Novel Influenza A H1N1 presented at Puerto Rico Neurology Society Annual Convention, 2009.

Influenza A H1N1 Immunization Campaign, AMPRE Annual Convention, 2009.
Prevention of Respiratory Viruses: RSV and Influenza, AAP Puerto Rico Chapter Meeting, 2009.
The Immunization Controversy: Myths and Realities, Neuroscience and Epilepsy Conference, Puerto Rico Neurology Society, 2008.
Adolescent Immunization, Puerto Rico Department of Health Immunization Conference, 2008
Immunization, The PR Style, presented at Puerto Rico Pediatric Society Annual Convention, 2007.
Influenza Prevention, presented at Immunization Annual Conference, Immunization Program, PR Department of Health. 2007.
Hepatitis A and B Prevention, Puerto Rico Pediatric Society Annual Convention, 2006.
Adult and Adolescent Immunization, Puerto Rico Department of Health, Immunization Conference, 2005.
Pediatric AIDS: Management of HIV Infected Child, Puerto Rico Department of Health, 1992, 1993, 1994.
Blood Transfusions in Pediatric Patients, Auxilio Mutuo Hospital Pediatric Meeting.

Diagnosis of Systemic Candidiasis Using Antigen Detection Tests, University of Puerto Rico, Graduate School of Microbiology, 1991.

Management of Bacterial Meningitis, University Pediatric Hospital, University of Puerto Rico School of Medicine,1991.

Toxic Shock Syndrome, Case Presentation, University Pediatric Hospital, UPR School of Medicine, 1990.

Pharmacokinetics of Theophylline, Abstract paper, University of Puerto Rico School of Medicine, 1985.

MELISSA MARZÁN RODRÍGUEZ

San Juan, Puerto Rico
melissa.marzan@salud.pr.gov

Educational History

2017-2019 **Post Doctoral Research Fellow**
University of Puerto Rico, Medical Sciences Campus
Northwestern University, Feinberg School of Medicine
Training Area: HIV prevention & Implementation Science
Sponsor: National Institutes on Minorities and Health Disparities (NIMHD)

2009-2015 **Doctor of Public Health in Epidemiology**
Honor Recognition
Ponce Health Sciences University
Public Health Program
Thesis: HIV Mortality and Survival Analysis in Puerto Rico for the period 2003-2011: A retrospective cohort study.

2007-2008 **Master of Public Health in Epidemiology**
University of Puerto Rico
Medical Sciences Campus Graduate School of Public Health
Thesis: Association between exposure to particulate matter from sand and gravel industries and respiratory diseases in residents of San Lorenzo, Puerto Rico: A cross sectional study.

2002-2006 **Bachelor in Arts in Anthropology**
Magna Cum Laude
University of Puerto Rico Río Piedras Campus

Professional Certification

2012-Present **Certified in Public Health (CPH)**
National Board of Public Health Examiners
NBPHE #1501

Awards

2018-Present **Diversity Scholar**
Adolescent Medicine Trials Network for HIV Prevention Interventions
Mentor: María Isabel Fernández, Ph.D.
Sponsor: Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)

2015 **Excellence Award**
Public Health Program
Ponce School of Medicine Foundation

2015	**Honor Recognition** Public Health Program DrPH Ponce Health Sciences University
2008-2010	**Trainee.** Research Supplements to Promote Diversity in Health-Related Research. Pre-Doctoral Qualitative Methods in Health Research Training Sponsor: National Institute of Mental Health (NIMH)
2002-2006	**Dean's List (top 5 % of the class)** University of Puerto Rico, Río Piedras Campus
2002-2006	**Scholar.** NIMH Career Opportunities in Research Program University of Puerto Rico, Río Piedras Campus

Academia

2015-Present	**Assistant Professor.** Public Health Program. Ponce Health Sciences University. Ponce, Puerto Rico Supervisor: Dr. Vivian Green
2019-Present	**Adjunct Assistant Professor.** School of Public Health. University of Puerto Rico, Medical Sciences Campus. San Juan, Puerto Rico Supervisor: Dr. Souhail Malavé
2015-Present	**Assistant Professor.** Clinical Psychology Program. Ponce Health Sciences University. Ponce, Puerto Rico Supervisor: Dr. Jose Pons Madera
2015-2019	**MPH-Epidemiology Program Coordinator.** Ponce Health Sciences University Public Health Programs. Ponce, Puerto Rico Supervisor: Dr. Vivian Green
2009	**Instructor.** University of Puerto Rico in Carolina. Department of Social Sciences and Criminal Justice. Carolina, Puerto Rico Supervisor: Prof. Juan Bonilla

Public Health Practice

2021-Present	**Chief Epidemiology Officer.** Puerto Rico Department of Health.
2020-Present	**Member.** COVID-19 Response in Puerto Rico. Health Taskforce of the South.
2018-Present	**Epidemiologist.** CDC Zika Response in Puerto Rico. Caduceus Healthcare, Inc & CDC San Juan Quarantine Station; Division of Global Migration & Quarantine. Supervisors: Carolina Luna Pinto, MPHE & Rebecca Ramos, JD
2013-2016	**Field Director.** PACTo Project: Enhanced HIV Care Access and Retention for Drug Users in San Juan, Puerto Rico. AIDS Surveillance. Puerto Rico Department of Health & Columbia University. Supervisors: Sandra Miranda, MPH; Paco Castellón, MPH

2008 & 2010-2013	**Field Supervisor.** AIDS Surveillance. National HIV Behavioral Surveillance-NHBS. Department of Health, San Juan, Puerto Rico Supervisor: Sandra Miranda, MPH
2008 & 2010-2013	**Ethnographer.** AIDS Surveillance. National HIV Behavioral Surveillance-NHBS. Department of Health, San Juan, Puerto Rico Supervisor: Sandra Miranda, MPH

Funded Research Projects

2020-Present	**Multiple Principal Investigator**. NIH-RADxUP Project #46.
2020-Present	**Multiple Principal Investigator**. MCC-PHSU U54 Pilot Project.
2018-Present	**Principal Investigator (Subaward).** Adolescents Medicine Trials Network- Pilot Project.
2020-Present	**Principal Investigator.** Puerto Rico Public Health Trust-COVID19 Emergency funds.
2018-Present	**Co-Investigator.** NIMHD-R21 Resilience Factors associated to Hurricanes Irma & Maria in the HIV healthcare services.
2017-2019	**Principal Investigator (Subaward).** NIMHD-U01 Supplement for Postdoctoral Training.

Scientific Activities

2008-Present	**Abstract Reviewer**. International AIDS Conference. International AIDS Society.
2019	**Co-Chair.** Session: 4G: Gaming, Grindr and getting the goods. International Conference on HIV Science. International AIDS Society.
2015-2016	**Abstract Mentor Programme**. International AIDS Conference. International AIDS Society.

Trainings

2021	**COVID-19 Equity Academy.** National Institutes on Minorities and Health Disparities (NIMHD).
2018	**Implementation Science & Dissemination Certification**. Training Institute for Dissemination and Implementation Research Health. National Institutes of Health (NIH).

Professional Affiliations

2011-Present	**Member.** American Public Health Association Member.
2018- Present	**Member.** International AIDS Society.
2007-2008	**Member.** Epidemiology and Biostatics Student Association.
2004-2006	**Member.** National Society of Collegiate Scholars.

Presentations

Marzan-Rodriguez, M., Jimenez, J., Morales, LM., Castro, E., Rosario, F., Velez, D., Ramos, A., Asencio, G., & Matos, J. (2021, February). *A community response for COVID-19 community transmission*. Poster presentation at: 2021 RCMI National Conference. Ponce Health Sciences University, RCMI-CEC.

Matos, J. , Ramos, A ., Rosa-Jiménez. A., Beauchamp-Lebrón, A., Morales, LM., Castro, E., Rosario, F., Velez, D., Asencio, G., **Marzan-Rodriguez**, M., & Jimenez, J. (2021, February). *Focus group discussion around community's health issues*. Poster presentation at: 2021 RCMI National Conference. Ponce Health Sciences University, RCMI-CEC.

Marzan-Rodriguez, M. (2021, February). *Epi-Net: Accelerating COVID-19 diagnostics among socially vulnerable communities.* Oral presentation at: 6th Menonita Medical Congress. San Juan, PR.

Marzán-Rodríguez, M., Christy, S., Brito, L., Gonzalez, C., Perez, A., Perez, C., Jimenez, J, & Vadaparampil, S. (2020, October). *Facilitators and Barriers to HPV Vaccination among Young Latino Men who have Sex with Men: A Systematic Review*. Presented as poster presentation at 2020 International Cancer Education Conference (virtual).

Marzán-Rodríguez, M., Rodríguez-Díaz, C., Jovet, G., Miranda De León, S., & Rivera-Malave, S. (2020). *Assessment of health-related outcomes in PLWH in Puerto Rico after the Hurricanes Irma and Maria*. E-poster: at 23rd International AIDS Conference. San Francisco, CA.

Marzán-Rodríguez, M., Mercedes, A., Rivera, A., & Rodríguez-Díaz, C. (2019). *Implementation Science framework to identify facilitators and barriers for an eHealth HIV prevention program for Spanish-speaking adolescents men who have sex with men in Puerto Rico.* Oral Presentation at 39th Research and Education Forum, Medical Sciences Campus, University of Puerto Rico. San Juan, Puerto Rico.

Santiago-Rodríguez, E., Martínez-Vélez, J.J., **Marzán-Rodríguez, M.**, & Rodríguez-Díaz, C. (2018). *Do ask and do tell: Providing capacity on LGBT Health to mental health providers*. Poster Presentation at American Public Health Association Conference. San Diego, California.

Marzán-Rodríguez, M., Santiago-Rodríguez, E., Malavé-Rivera, S., Vargas-Molina, R., & Rodríguez-Díaz, C. (2018). *HIV response in the midst of natural disasters and a humanitarian crisis: The case of Puerto Rico*. Poster Presentation at 22nd International AIDS Conference. Amsterdam, Netherlands.

Luna-Pinto, C., **Marzán-Rodríguez, M.**, Fonseca-Ford M., Prue, C., Hernandez-Burgos, J., Olano, H.A., Marrero-Padilla, C., & Rivera-Garcia, B. (May, 2017). *Evaluation of Zika health messages at ports of entry in Puerto Rico*. Poster Presentation at the International Society of Travel Medicine Conference. Barcelona, Spain.

Castellon, P., Cardenas, G., **Marzán-Rodríguez, M.**, Miranda-De León, M., Santana-Bagur, J., Rodriguez, A., Feaster, D., & Metsch, L. (November, 2016). *HIV care outcomes among injectors and non-injectors in San Juan, Puerto Rico*. Poster Presentation at American Public Health Conference. Denver, Colorado.

Marzán-Rodríguez, M., Castellon, P., Miranda-De León, M., Vargas-Vidot, J., Feaster, D., Santana-Bagur, J., & Metsch, L. (May, 2016). *Proyecto PACTo: Implementing and Evaluating a Community-level, Structured Approach to Enhance HIV Care Access and Retention for Substance Users in San Juan, Puerto Rico.* Poster Presentation at 5th Puerto Rico Public Health Conference. San Juan, Puerto Rico.

Marzán-Rodríguez, M., Zavala, D.E., Orengo, J.C., Varas-Díaz, N., & Miranda de León, S. (August, 2015). *Mortality analysis in people diagnosed with HIV/AIDS in Puerto Rico from 2003-2011.* Oral Presentation at 3th International Conference on Epidemiology & Public Health. Valencia, Spain.

Marzán-Rodríguez, M., Zavala, D.E., Orengo, J.C., Varas-Díaz, N., & Miranda de León, S. (August, 2015). *Survival analysis in people diagnosed with HIV/AIDS in Puerto Rico from 2003-2011.* Poster Presentation at 3th International Conference on Epidemiology & Public Health. Valencia, Spain.

Marzán-Rodríguez, M., & Varas-Díaz, N. (October, 2012). *Definición y manejo de estigma hacia el VIH/SIDA post intervención de reducción.* Oral Presentation at V Congreso Iberoamericano de Análisis Cualitativo en Salud. Lisboa, Portugal.

Betancourt-Díaz, E., **Marzán-Rodríguez, M.**, & Varas-Díaz, N. (October, 2012). *Experiencias de estudiantes de medicina durante intervención de reducción de estigma hacia el VIH/SIDA.* Oral Presentation at V Congreso Iberoamericano de Análisis Cualitativo en Salud. Lisboa, Portugal.

Nawab, S., Vazquez, J., Acevedo, M., & **Marzán-Rodríguez, M.** (November 18-21, 2011). *Low CD 4 T cell count in uninfected HIV exposed infants in association with anti-retroviral therapy exposure during pregnancy in San Juan Hospital.* Oral Presentation at 2011 HIV Caribbean Conference. Nassau, Bahamas.

Cintrón-Bou, F., **Marzán-Rodríguez, M.** & Varas-Díaz, N. (November 18- 21, 2011). *Emotions and HIV related stigma: Impacting health professionals in Puerto Rico.* Oral Presentation at 2011 HIV Caribbean Conference. Nassau, Bahamas.

Marzán-Rodríguez, M., & Varas, Díaz, N. (July, 2011). *Understanding socio- structural factors in the HIV epidemic as part of a stigma reduction strategy with medical students in Puerto Rico.* ePoster at International AIDS Society, 2011. Rome, Italy.

Nawab, S., Acevedo, M., & **Marzán-Rodríguez, M.** (July, 2011). *Low CD 4 T cell count in uninfected HIV exposed infants in association with anti-retroviral therapy exposure during pregnancy in San Juan Hospital.* ePoster at International AIDS Society, 2011. Rome, Italy.

Nawab, S., Vázquez, J. L., Acevedo, M., & **Marzán-Rodríguez, M.** (September 25, 2010). *Low CD 4 T cell count in uninfected HIV exposed infants in association with anti-retroviral therapy exposure during pregnancy in San Juan Hospital.* Oral Presentation at Convención Anual de Pediatría de Puerto Rico. San Juan, Puerto Rico.

Marzán-Rodríguez, M., & Varas, Díaz, N. (September 9-11, 2010). *Factores socio-estructurales y VIH: Experiencias de estudiantes de medicina en una intervención de*

reducción de estigma de VIH/SIDA. Oral Presentation at IV Congreso Iberoamericano de Investigación Cualitativa en Salud. Fortaleza, Brasil.
Marzán-Rodríguez, M., & Varas-Díaz, N. (May 13-15, 2010) *Using qualitative methods to assess HIV/AIDS stigma reduction among medical students.* Oral Presentation at 27th Annual Qualitative Analysis Conference: Social Pragmatism as a Conceptual Foundation. Brantford, Ontario, Canadá.

Pascual Marrero, A.M., Pascual Marrero, J., **Marzán-Rodríguez, M.**, et al. (November, 2009). *Association between exposure to particulate matter from sand and gravel industries and respiratory diseases in residents of San Lorenzo, Puerto Rico: A cross sectional study.* Poster Presentation at 137th APHA Annual Meeting. Philadelphia, Pennsylvania.

Pascual Marrero, A., et al. (April, 2009). *Crossectiontal study on the association between exposure to particulate matter and respiratory diseases among residents from the San Lorenzo municipality, Puerto Rico, 2008.* XXIX Research and Education Forum, Medical Sciences Campus, University of Puerto Rico. San Juan, Puerto Rico.

Marzán-Rodríguez, M., Cintrón Bou, F., & Varas-Díaz, N. (June, 2009). *Más que responsabilidad individual: Estigma, VIH y factores socio-estructurales.* Oral Presentation at XXII Congreso Interamericano de Psicología. Ciudad de Guatemala, Guatemala.

Malavé Rivera, S., **Marzán-Rodríguez, M.**, & González Arias, R. (June, 2009). *El rol del cuerpo en la estigmatización del VIH/SIDA.* Oral Presentation at XXII Congreso Interamericano de Psicología. Ciudad de Guatemala, Guatemala.

Marzán-Rodríguez, M., & Varas-Díaz, N. (May, 2008). *Un acercamiento cualitativo a las emociones y el estigma: El VIH/SIDA en Puerto Rico como ejemplo.* Oral Presentation at III Congreso Iberoamericano de Investigación Cualitativa en Salud. San Juan, Puerto Rico.

Marzán-Rodríguez, M., & Varas-Díaz, N. (June, 2006). *A mixed method approach for studying emotions and AIDS stigma among health profession students in Puerto Rico.* Poster Presentation at Society of Social History of Medicine Annual Conference 2006, Practices and representations of health: Historical perspectives. Coventry, Birmingham, England.

Marzán-Rodríguez, M., & Varas-Díaz, N. (November, 2005). *Emotions: An obstacle or facilitator in the stigmatization of HIV/AIDS in Puerto Rico.* Oral Presentation at the COR Colloquium 2005. Atlanta, Georgia.

Marzán-Rodríguez, M., & Barker, Judith C. (August, 2005). *Gender Differences in Older Mexican-Americans Smoking Behaviors.* Poster session at Summer Research Training Program at the University of California, San Francisco. USA.

Marzán-Rodríguez, M., & Barker, Judith C. (August, 2005). *Gender Differences in Older Mexican-Americans Smoking Behaviors.* Oral presentation at Summer Research Training Program at the University of California, San Francisco. USA.

Marzán-Rodríguez, M., Ruiz-Torres, Y., & Varas-Díaz, N. (June, 2005). *Emociones, diferencia y estigma: ¿Qué nos evoca el VIH/SIDA a los/as profesionales de la salud?* Poster session at XXX Congreso Interamericano de Psicología de la Sociedad Interamericana de Psicología, Buenos Aires, Argentina.

Marzán-Rodríguez, M., Rosselló, J., Jiménez, M.I., & Sáez, E. (November, 2004). *The Relationship between socioeconomic status, self-care and metabolic control in Puerto Rican youth with type 1diabetes.* Poster session at COR Colloquium, November 2004. San Juan, Puerto Rico.

Published Manuscripts in Peer Review Journals

Marzán-Rodríguez, M. (In Press). Una mirada desde los Determinantes Sociales de la Salud a la epidemiología del COVID-19 en Puerto Rico. [In Spanish]. *Revista Salud y Sociedad.*

Marzán-Rodríguez, M., Lugo-Hernández, E.A., Morales, L.M., & Martinez, I. (Manuscript Preparation). Fortaleciendo alianzas para enfrentar las Emergencias de Salud Pública en Puerto Rico: Una mirada desde la epidemiología social. Memorias de la Convención Anual de la Asociación de Psicología de Puerto Rico 2020.

Rodríguez-Díaz, C., **Marzán-Rodríguez, M.,** Mustanski, B., Macapagal, K., & Malavé-Rivera, S. (Under review). Ethical considerations for research with young sexual minorities and opportunities to reduce HIV disparities in Puerto Rico. *Puerto Rico Health Sciences Journal.*

Marzán-Rodríguez, M., Rodríguez-Díaz, C., &, Mustanski, B. (2020). Health HIV prevention among Spanish-speaking adolescent men who have sex with men: A systematic review of the literature and recommendations for the development of interventions. *Sexuality Research & Social Policy.*

Marzán-Rodríguez, M., et al. (2020). Syndromic Surveillance in Puerto Rico during COVID19 response: An alternative approach to scarce molecular testing. *American Journal of Public Health.* DOI: 10.2105/AJPH.2020.305805.

Rodriguez-Diaz, C. E., Guilamo-Ramos, V., Mena, L., Hall, E., Honermann, B., Crowley, J. S., Baral, S., Prado, G. J., **Marzan-Rodriguez, M.,** Beyrer, C., Sullivan, P. S., & Millett, G. A. (2020). Risk for COVID-19 infection and death among Latinos in the United States: Examining heterogeneity in transmission dynamics. *Annals of epidemiology,* S1047-2797(20)30267-2. Advance online publication. https://doi.org/10.1016/j.annepidem.2020.07.007

Marzán-Rodríguez, M., Zavala, D.E., Orengo, J.C., Varas-Díaz, N., Miranda de León, S., Acevedo-Diaz, E. (2018). Survival analysis in people diagnosed with HIV/AIDS in Puerto Rico from 2003-2011 [In Spanish]. *Revista Puertorriqueña de Medicina y Salud Pública,* 66; 8-14.

Cintrón Bou, F., Varas Díaz, N., **Marzán-Rodríguez, M.**, & Neilands, T.B. (2016). Experiencias de estudiantes de medicina: Intervención para reducir estigma relacionado al VIH/SIDA. [In spanish]. *Revista Interamericana de Psicología*, 50 (*1*): 137-148.

Marzán-Rodríguez, M., Varas Díaz, N, & Neilands, T.B. (2015). Qualitative contributions to a randomized controlled trial addressing HIV/AIDS-Stigma in medical students. *The Qualitative Report*, 20 (*12*): 2012-2024.

Marzán-Rodríguez, M., Rodríguez-Madera, S., & Varas Díaz, N. (2014). Stigma and homophobia: Persistent challenges for HIV prevention among young MSM in Puerto Rico.

Revista de Ciencias Sociales, 26 (2013): 50-59.

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Varas Díaz, N., Neilands, T.B., Cintrón Bou, F., Santos-Figueroa, A., **Marzán-Rodríguez, M.**, & Marques, D. (2013). Religion and HIV/AIDS stigma in Puerto Rico: A cultural challenge for training future physicians. *Journal of International Association of Physicians AIDS Care, 12 (2).*

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Varas Díaz, N., & **Marzán-Rodríguez, M.** (2007). The emotional aspect of AIDS stigma among health professionals in Puerto Rico. *AIDS Care.* Volume *19*(10). pp. 1247-1257.

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Other Publications

Marzán-Rodríguez, M., & Morales, LM. (2021). Las variantes del COVID y sus implicaciones en la salud pública. [Newspaper: January 28, 2021]. El Nuevo Día: https://www.elnuevodia.com/opinion/punto-de-vista/las-variantes-del-covid-y-sus-implicaciones-en-la-salud-publica/

Marzán-Rodríguez, M. (2020). La nueva ruta contra el COVID-19. [Newspaper: December 12, 2020]. El Nuevo Día: https://www.elnuevodia.com/opinion/punto-de-vista/mascarillas-distanciamiento-lavado-de-manos-y-vacunas-la-nueva-ruta/

Marzán-Rodríguez, M. (2020). COVID-19: "Todavía estamos lejos de que la pandemia se vuelva endemia". [Note: November 13, 2020]. MedScape: https://espanol.medscape.com/verarticulo/5906179

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Marzán-Rodríguez, M. (2020). La información científica del COVID-19. [Newspaper: March 9, 2020]. El Nuevo Día: https://www.elnuevodia.com/opinion/punto-de-vista/la-informacion-cientifica-sobre-covid-19/

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vista/las-implicaciones-del-diagnostico-tardio-del-covid-19/

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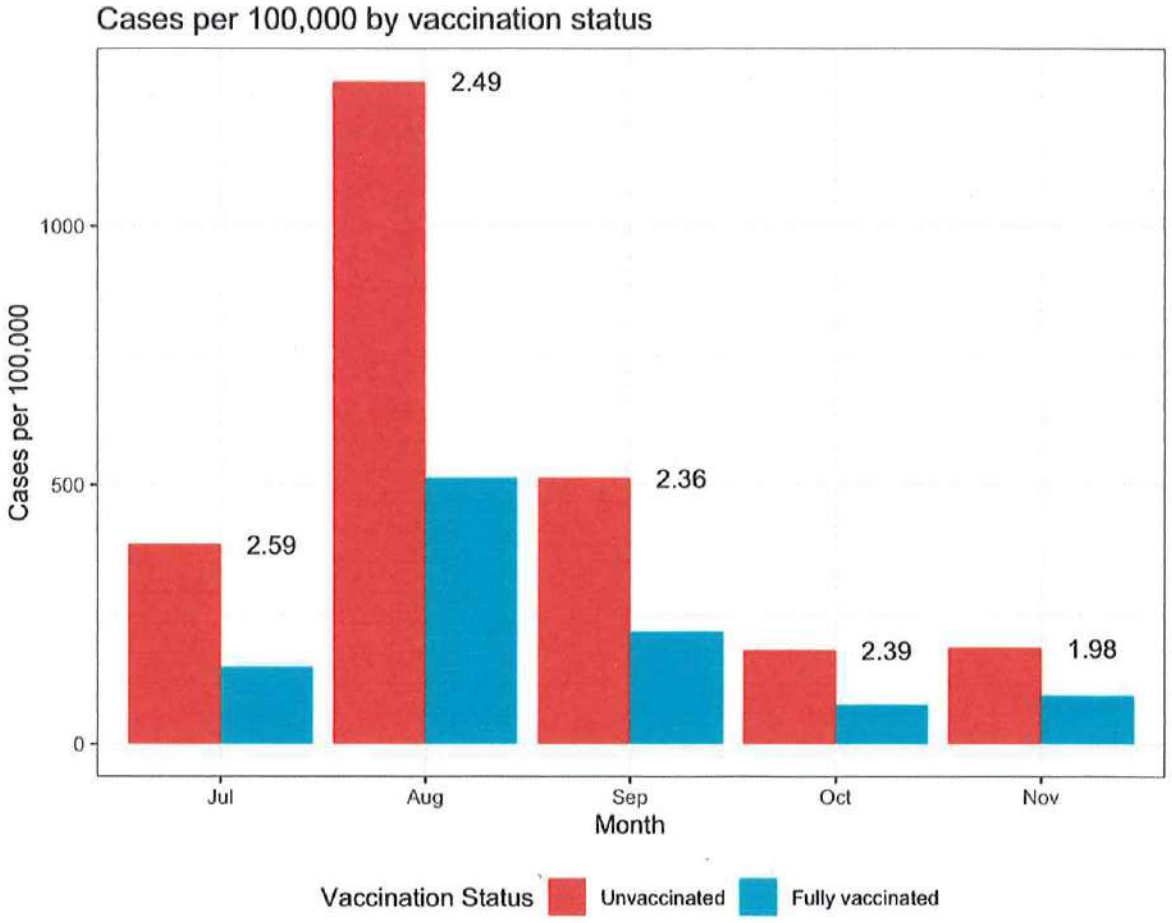
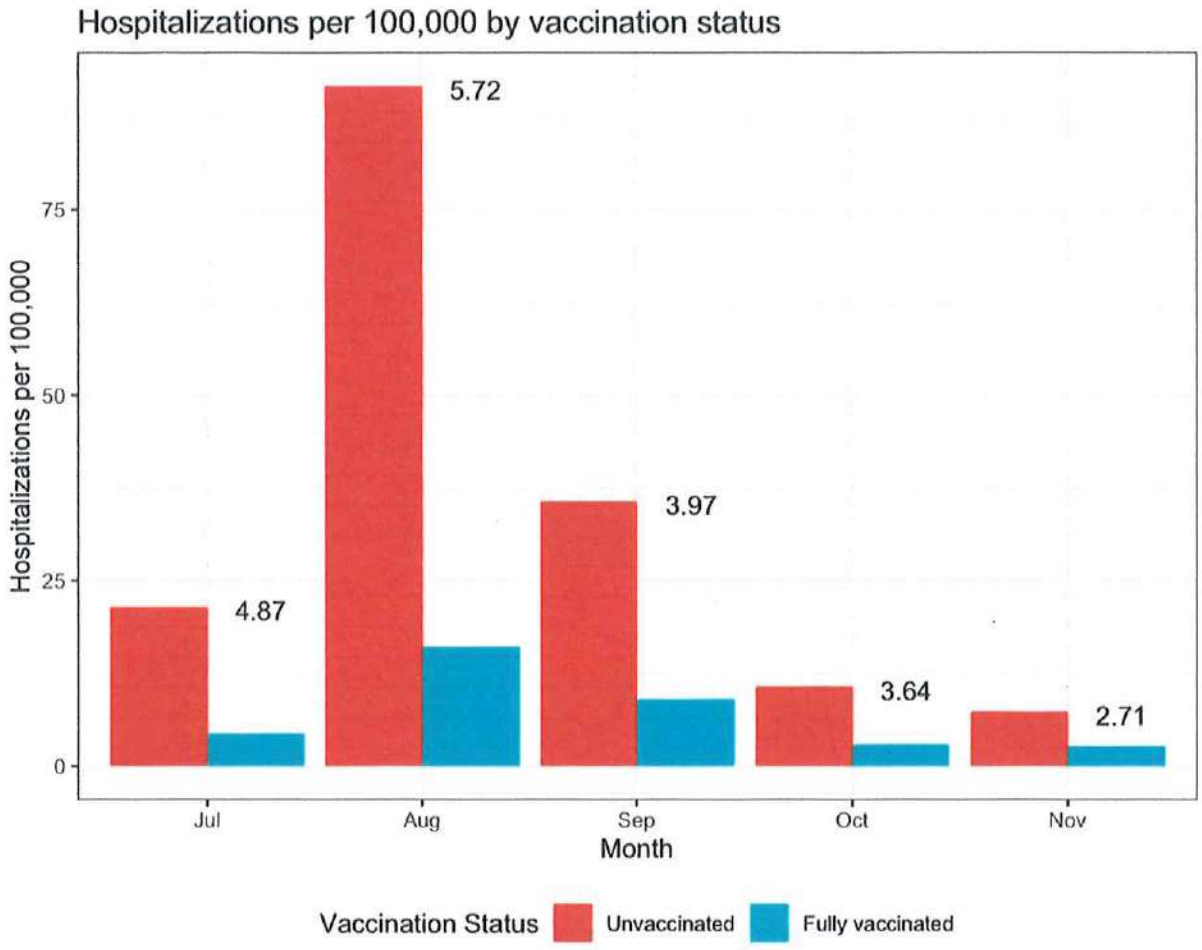
Available upon request.

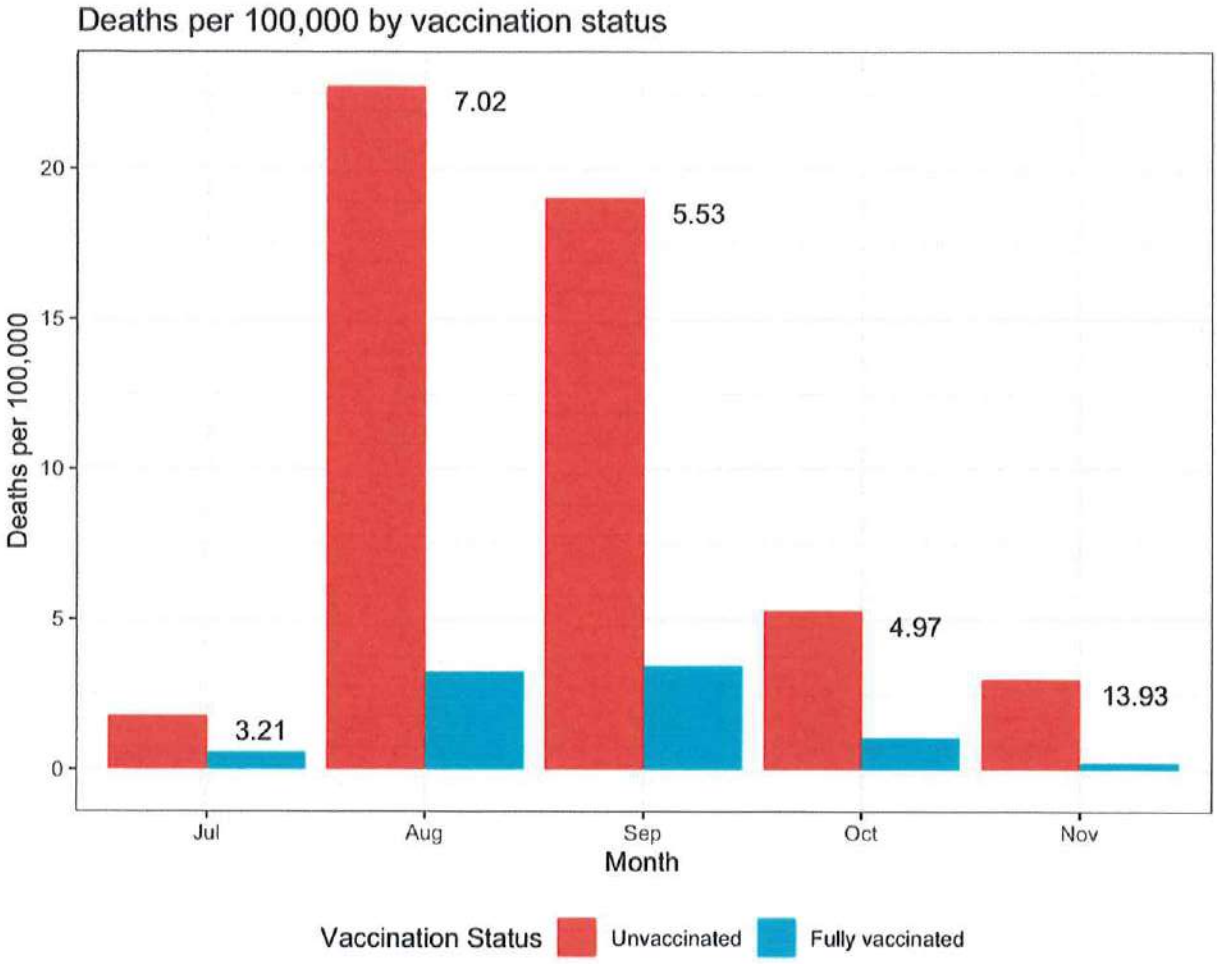


Source: Puerto Rico Department of Health. COVID-19 Cases by vaccination status for the July to November 2021 period. Data as of: December, 9, 2021.



The number on the columns represent the relative risk of the event for unvaccinated people in comparison to those fully vaccinated. Unvaccinated refers to persons who had not started their vaccination routine at the moment of diagnosis. Fully vaccinated are persons who completed their vaccination routine and were diagnosed at least 14 days after their last dose.

Source: Puerto Rico Department of Health. COVID-19 Hospitalizations by vaccination status for the July to November 2021 period. Data as of: December, 9, 2021.



The number on the columns represent the relative risk of the event for unvaccinated people in comparison to those fully vaccinated. Unvaccinated refers to persons who had not started their vaccination routine at the moment of diagnosis. Fully vaccinated are persons who completed their vaccination routine and were diagnosed at least 14 days after their last dose.

Source: Puerto Rico Department of Health. COVID-19 Deaths by vaccination status for the July to November 2021 period. Data as of: December, 9, 2021.

BIOGRAPHICAL SKETCH

NAME: Irizarry, Rafael A.

eRA COMMONS USER NAME: ririzarr

POSITION TITLE: Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
University of Puerto Rico, Río Piedras	B.S.	05/1993	Mathematics
University of California, Berkeley	Ph.D.	05/1998	Statistics

A. Personal Statement

I have dedicated my career to helping biomedical researchers better interpret their data through the development of statistical tools and software. I have focused on data generated by high-throughput genomic technologies. The tools I develop often arise from close collaborations focused on data-driven discovery. Some of these collaborations have led to important basic biology insights such as discoveries related to CpG island shores. Generally applicable statistical ideas often emerge from this collaborative work and in these cases I have transformed these ideas into rigorous data analysis pipelines disseminated as open source software. To do this systematically, I co-founded, and currently co-lead, the Bioconductor project, which provides one the most widely used software packages for genomics data analysis. The statistical methodologies and software that I have developed, and made available through Bioconductor, include highly cited work on signal processing of high-throughput transcription data, correcting for systematic errors in protein binding data, and statistical inference for the detecting differentially methylated DNA regions. I am also deeply committed to training our next generation of data analysts. In this endeavor, I have mentored dozens of undergraduate students, graduate students, postdoctoral fellows, and junior faculty. I have received several awards for my NIH funded work including the COPSS presidents' Award, arguably the statistical profession's most prestigious award, and the Benjamin Franklin Award in the Life Sciences.

1. Nakayama RT, Pulice JL, Valencia AM, McBride MJ, McKenzie ZM, Gillespie MA, Ku WL, Teng M, Cui K, Williams RT, Cassel SH, Qing H, Widmer CJ, Demetri GD, **Irizarry RA**, Zhao K, Ranish JA, Kadoch C. (2017) SMARCB1 is required for widespread BAF complex-mediated activation of enhancers and bivalent promoters. *Nat Genetics* 2017 Nov;49(11):1613-1623.
2. Korthauer K, Chakraborty S, Benjamini Y, **Irizarry RA** (2018) Detection and accurate false discovery rate control of differentially methylated regions from whole genome bisulfite sequencing. *Biostatistics.*
3. Love MI, Hogenesch JB and **Irizarry RA** (2016) Modeling of RNA-seq fragment sequence bias reduces systematic errors in transcript abundance estimation. *Nature Biotechnology* Dec;34(12): 1287-1291. PMCID: PMC5143225.
4. Huber W, Carey VJ, Gentleman R, Anders S, Carlson M, Carvalho BS, Bravo HC, Davis S, Gatto L, Girke T, Gottardo R, Hahne F, Hansen KD, **Irizarry RA**, Lawrence M, Love MI, MacDonald J, Obenchain V, Oleś AK, Pagès H, Reyes A, Shannon P, Smyth GK, Tenenbaum D, Waldron L, Morgan M (2015) Orchestrating high-throughput genomic analysis with Bioconductor. *Nature Methods* Feb; 12(2): 115-21. PMCID: PMC4509590.

B. Positions and Honors

Positions and Employment

1998 – 2004 Assistant Professor, Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore (JHBSPH), Baltimore Maryland
2004 – 2007 Associate Professor, Department of Biostatistics, JHBSPH, Baltimore, Maryland
2007 – 2013 Professor, Department of Biostatistics, JHBSPH, Baltimore, Maryland
2013 –2018 Professor, Department of Biostatistics and Computational Biology, Dana -Farber Cancer Institute
2013 – Professor, Department of Biostatistics, Harvard School of Public Health

2018 – Professor and Chair, Department of Data Science, DFCI

Honors

2001	American Statistical Association Noether Young Scholar Award for researcher, younger than 35 years of age, who has significant research accomplishments in nonparametric statistics.
2004	ASA Outstanding Statistical Application Award
2006	ASA Youden Award in Inter-Laboratory Testing
2009	COPSS President's Award presented to a young member of the statistical community in recognition of outstanding contribution to the profession.
2009	Mortimer Spiegelman Award which recognizes a statistician age 40 years or younger who has made outstanding contributions to public health statistics.
2009	American Statistical Association Fellow
2012	Myrto Lefkopoulou Distinguished Lecturer Award
2010-2014-	NIH Genomics, Computational Biology & Technology NIH (GCAT) Study Section member
2017-	Chair of Genomics, Computational Biology and Technology NIH Study Section
	Benjamin Franklin Award in the Life Sciences

C. Contributions to Science

I began my career as an independent researcher in a Biostatistics Department. This came at an opportune moment since at the time Biology was changing from a data-poor discipline to a data-intensive one. Much of my work is motivated by collaborations with researchers who, for the first time, were collecting large amounts of data using high-throughput technologies. My major contributions to science relate to statistical methods and software that permit biological discoveries from the complex datasets generated with high-throughput technologies. Below I list my five major contributions.

Signal Processing for Transcriptome Data

High-throughput technologies changed the way we measure gene expression from spotting black dots on a piece of paper or extracting a few numbers to sifting through tens of thousands of numbers. Biologists went from using their eyes or simple summaries to analyzing thousands (and now millions) of measurements per sample. Complexity was exacerbated by unpolished technologies that made measurements much noisier than anticipated: first with microarrays and then with next generation sequencing. My first major scientific contribution was the development of statistical methodology and software for the normalization and signal processing of raw microarray data. In particular, the Robust Multiarray Analysis (RMA) method **[1]** has become an indispensable standard in the field. The method has several advantages in the context of the most common challenges faced by biologists [2]. This processing method has become a standard in the field and the two referenced papers [1,2] combine for well over 10,000 citations. More recently, RNA-seq technology became more widely used among my collaborators. Soon after commencing work with this data type, we discovered that this technology was also prone to systematic error. Specifically, GC-content appeared to have a strong unwanted effect on the probability of observing a given fragment. We developed statistical methodology to account for this systematic bias [3] and soon after, in collaboration with computer scientists, developed a fast and efficient software implementation [4]. This software tool is currently one of most widely used quantification methods for RNA-Seq data [4].

[1] **Irizarry RA**, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP (2003) Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 4:249-264. (9,079 citations as of April 21, 2018 according to Google Scholar).

[2] **Irizarry RA**, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP (2003) Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Research 31:e15. (4,745 citations).

[3] Love MI, Hogenesch JB and **Irizarry RA** (2016) Modeling of RNA-seq fragment sequence bias reduces systematic errors in transcript abundance estimation. Nature Biotechnology Dec;34(12): 1287-1291. PMCID: PMC5143225. (18 citations)

[4] Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. (2017) Salmon provides fast and bias-aware quantification of transcript expression. Nat Methods Mar 6. PMCID: PMC5600148. (207 citations)

Epigenetics

I have leveraged my data science skills to help make important biological discoveries in the field of epigenetics. My experience developing quantitative methods and software for improving high-throughput measurements provides a great advantage for my collaborators. One of my collaborators was particularly interested in using these technologies to understand a molecular outcome never before observed: whole genome methylation patterns. Previously, the field was focused on a specific part of the genome denoted CpG islands. I therefore helped design a technology and statistical methodology that leveraged my design to extract signals. While exploring the data and fine-tuning my methodology, I discovered that tissue and cancer specific changes in DNA methylation were more common in regions just outside of CpG islands, regions that I named shores. The paper describing this discovery [1], has led to improvements in commercial products and changed the way methylation data are analyzed. I later led the effort to extend the statistical analysis performed to make this discovery so that it was generally applicable and published a method that is now widely used to detect differentially methylated regions [2]. Once our collaborators and a large part of the research commenced using measurement techniques based on next generation, we started an extension of our widely used method that was appropriate for this technology. This method was very recently published [3]. Finally, another important contribution of the field of epigenetics is described in a paper pointing out the importance of accounting for cellular heterogeneity is critical in epigenome-wide association studies [4].

[1] **Irizarry RA**, Ladd-Acosta C, Wen B, Wu Z, Montano C, Onyango P, Cui H, Gabo K, Rongione M, Webster M, Ji H, Potash J, Sabunciyan S, Feinberg AP (2009) The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. Nature Genetics. 41(2):178-86. PMCID:PMC2729128. (1,716 citations).

[2] Jaffe AE, Murakami P, Lee H, Leek JT, Fallin MD, Feinberg AP, **Irizarry RA** (2012) Bump hunting to identify differentially methylated regions in epigenetic epidemiology studies. International Journal of Epidemiology 41(1):200-9. PMCID:PMC3304533. (279 citations).

[3] Korthauer K, Chakraborty S, Benjamini Y, **Irizarry RA** (2018) Detection and accurate false discovery rate control of differentially methylated regions from whole genome bisulfite sequencing. *Biostatistics.*

[4] Jaffe AE, **Irizarry RA** (2014) Accounting for cellular heterogeneity is critical in epigenome-wide association studies. Genome Biology Feb 4; 15(2):R31. PMCID: PMC4053810. (394 citations)

Open Source Software for Genomics

While addressing data challenges such as those described in the previous sections, a group of statisticians noticed that we were tackling many common computational challenges. We realized that a collaborative effort would be much more efficient. I was one of the first developers of the Bioconductor project [1] and continue as one of the leaders. Bioconductor is an open-source, open-development software project for the analysis and comprehension of high-throughput data in genomics and molecular biology. The project aims to enable interdisciplinary research, collaboration, and rapid development of scientific software, and hosts some of the most widely used software for Genomics. Based on the statistical programming language R, Bioconductor comprises over 1,000 interoperable packages contributed by a large, diverse community of scientists. Other than being part of the leadership, my main contribution to this project was the development of software implementing the signal processing methods described in the previous section [2]. An important feature of our software is that we not only provided ready-to-use algorithms, but also a flexible infrastructure for others to develop their own methods. As new technologies arise, we have continued to develop widely used software packages, for example DNA methylation data [3]. The specific packages I help create or maintain were downloaded from well over 30,000 unique IPs just this last year. I also continue to develop and support Bioconductor as described in a recent publication [4].

[1] Gentleman RC, Carey VJ, Bates DJ, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, **Irizarry RA**, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth GK, Tierney L, Yang YH, Zhang J (2004) Bioconductor: Open software development for computational biology and bioinformatics. Genome Biology 5:R80. (5,874 citations).

[2] Gautier L, Cope LM, Bolstad BM, **Irizarry RA** (2004) affy - An R package for the analysis of Affymetrix GeneChip data at the probe level. Bioinformatics. 20: 307-315. (1,597 citations).

[3] Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, **Irizarry RA** (2014) Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics 15;30(10):1363-9. PMCID:PMC4016708. (671 citations).

[4] Huber W, Carey VJ, Gentleman R, Anders S, Carlson M, Carvalho BS, Bravo HC, Davis S, Gatto L, Girke T, Gottardo R, Hahne F, Hansen KD, Irizarry RA, Lawrence M, Love MI, MacDonald J, Obenchain V, Oleś AK, Pagès H, Reyes A, Shannon P, Smyth GK, Tenenbaum D, Waldron L, Morgan M (2015) Orchestrating high-throughput genomic analysis with Bioconductor. Nat. Methods Feb; 12(2): 115-21. PMCID: PMC4509590. (681 citations)

Statistical Methodology for High-throughput Data

I started my career working on gene expression microarrays. During the following years, I leveraged the knowledge gained from this experience to develop solutions for other applications and data types. The resulting publications and software tools have had substantial impact in genomics. Examples not cited above describe the importance of accounting for batch effects [1], normalization approaches for other technologies [2], accounting for missing data in single cell RNA-seq application [3] and removing GC-content bias from ChIP-Seq data.

[1] [Leek JT, Scharpf RB, Bravo HC, Simcha D, Langmead B, Johnson WE, Geman D, Baggerly K, **Irizarry RA** (2010) Tackling the widespread and critical impact of batch effects in high-throughput data. Nat Rev Genet. 2010 11(10):733-9. PMCID:PMC3880143. (891 citations).

[2] Hansen KD, **Irizarry RA**, Wu Z (2012) Removing technical variability in RNA-seq data using conditional quantile normalization. Biostatistics 13(2):204-16. PMCID:PMC3297825. (259 citations).

[3] Hicks SC, Townes FW, Teng M, Irizarry RA. (2017) Missing data and technical variability in single-cell RNA-sequencing experiments. Biostatistics. 2017 Nov 6. (47 citations)

[4] Teng M, Irizarry RA. (2017) Accounting for GC-content bias reduces systematic errors and batch effects in ChIP-seq data. Genome Res. 2017 Nov;27(11):1930-1938. (2 citations)

Complete list of published work: http://www.ncbi.nlm.nih.gov/pubmed/?term=irizarry+RA

D. Research Support

Ongoing Research Support

U41 HG004059 (Morgan) 09/28/06 – 02/28/20
NIH/NHGRI (Subcontract: Roswell Park Cancer Institute)
Bioconductor: an Open Computing Resource for Genomics
The goal of this project is to develop infrastructure and software for the preprocessing of microarray and second generation sequencing data. Role: Co-Investigator

R01GM083084 (Irizarry) 09/24/07 – 06/30/20
NIH/NIGMS
Preprocessing and Analysis Tools for Contemporary Microarray Applications
To develop statistically rigorous procedures for five microarray applications with a rapidly growing user base: high-throughput genotyping, whole-genome chromosomal abnormality detection, discovering promoter binding sites with ChIPchip technology, high-throughput detection of methylated sites, & alternative splicing detection.

R01HG005220 (Irizarry) 08/11/10 – 02/28/19
NIH/NHGRI
Overcoming Bias and Unwanted Variability in Next Generation Sequencing
The goals of this project are to develop statistical methods for RNA transcript estimation that are robust to sequencing artifacts, develop statistical and computational tools that estimate and account for heterogenous cell composition in DNA methylation data, develop statistical and computational tools for unbiased quantification in microbial community 16S rRNA gene sequencing studies, and develop methods that account for protocol-induced bias in genome-wide enrichment scans.

R25GM114818 (Irizarry) 09/29/14 – 08/31/18 (NCE)
NIH/NIGMS (Subcontract: HSPH)
HarvardX Biomedical Data Science Curriculum
We aim to develop an open online biomedical data science curriculum, deliver a sustainable curriculum via the edX platform, and disseminate knowledge gained from preparing and teaching this curriculum.

R25MD010399 (Garcia-Arraras) 09/25/15 – 06/30/20

NIH/NIMHD (Subcontract: Univ. of Puerto Rico)
Increasing Diversity in Interdisciplinary BD2K (IDI-BD2K)
This proposal addresses the need for training the next generation of Hispanic scientists in Biomedical Big Data Research to enhance the diversity of the scientific community in this biomedical area. Role: Co-Investigator

U01CA214846 (Carey) 05/01/17 – 04/30/20
NIH/NCI
Accelerating Cancer Genomics with Cloud-scale Bioconductor
This proposal takes a design and architecture approach from a widely used project for analyzing general data arising in genome-scale biology, and adapts it to new NCI-supported cloud-based data archives and analysis environments. The proposal will accelerate identification of sources of variation of tumor responsiveness to treatment and will aid physicians in devising personalized antitumor strategies. Role: Co-Investigator

Completed Research Support

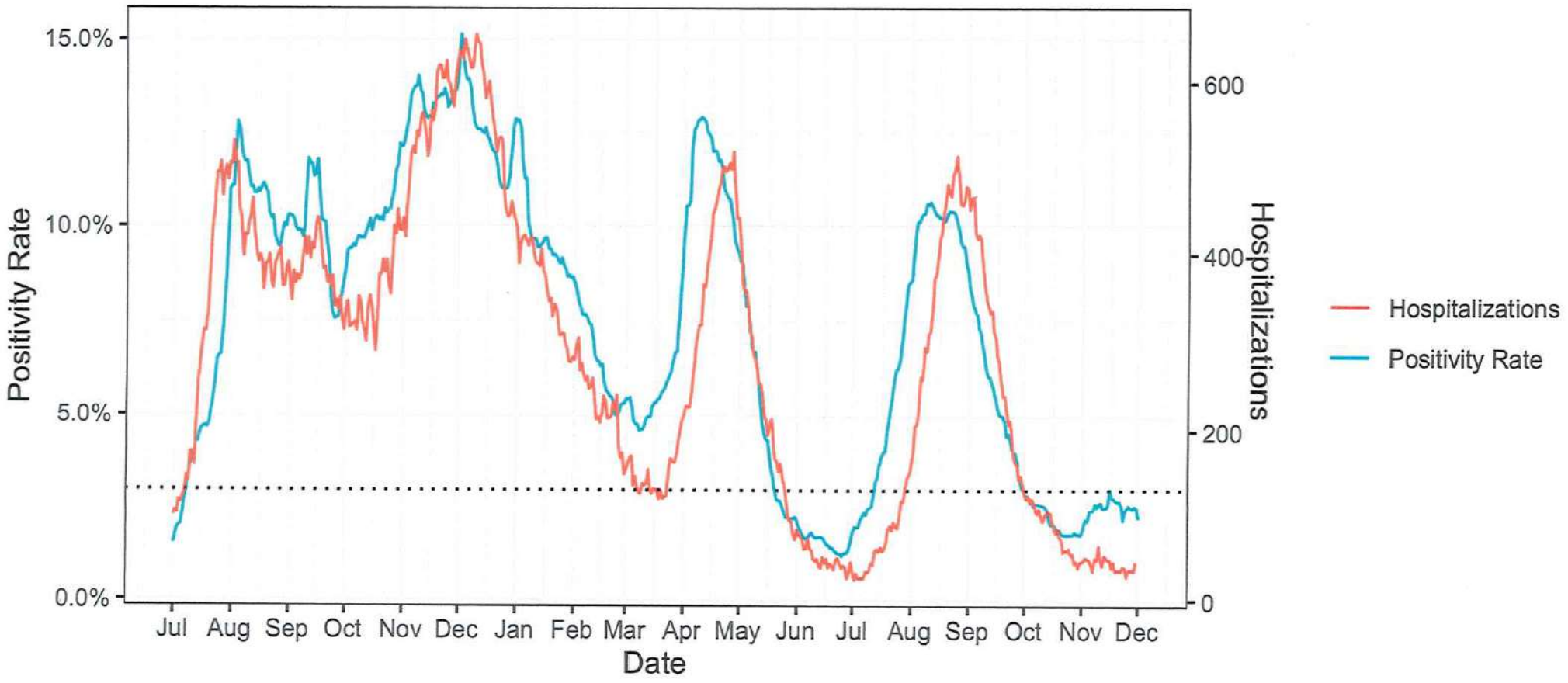
U24HG009446 (Weng) 02/01/17 – 01/31/21
NIH/NHGRI (Subcontract: UMass Medical Center)
EDAC: ENCODE Data Analysis Center
We will establish a data analysis center for the ENCODE project, with the goal of performing quality control and integrative analysis of ENCODE data and building the ENCODE Encyclopedia. Role: Co-Investigator

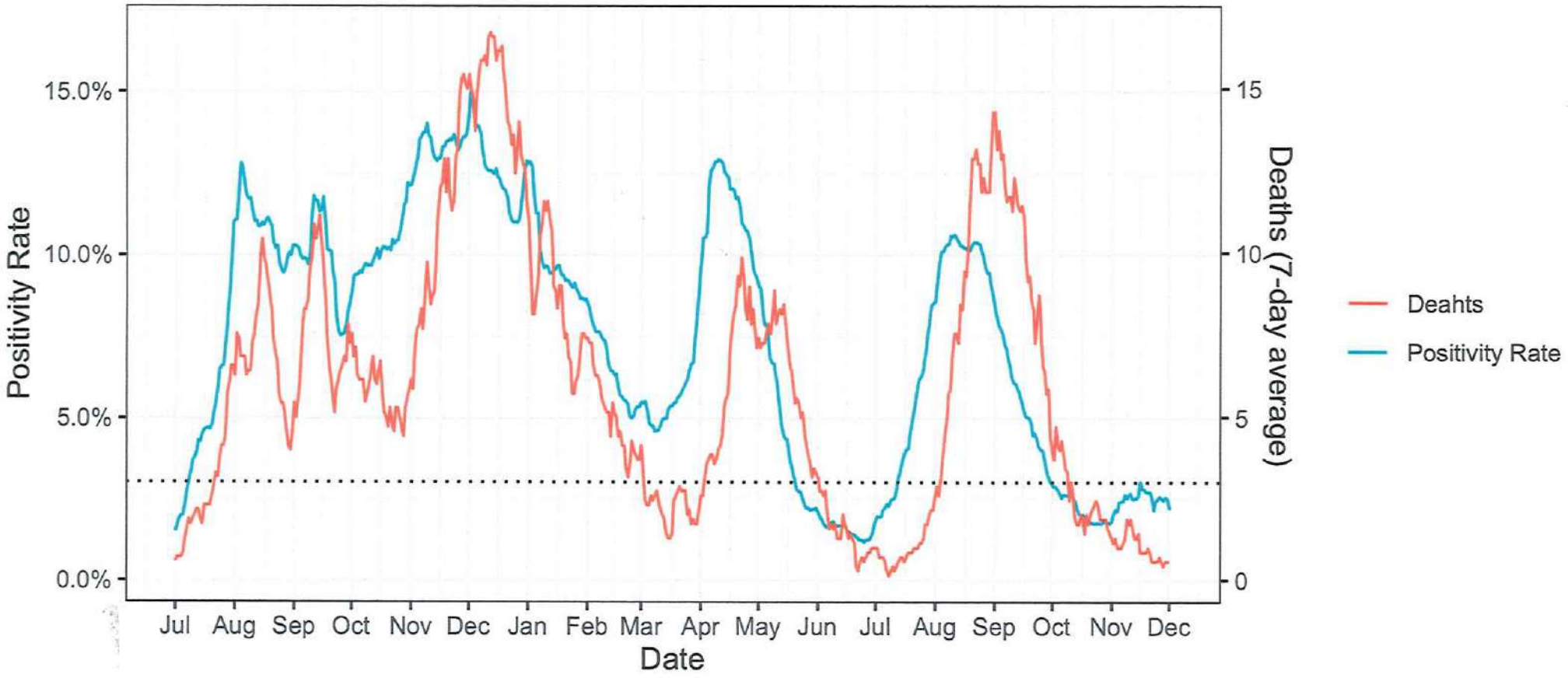
R01GM103552 (Irizarry) 08/01/12 – 07/31/17
NIH/NCR
Software for the statistical analysis of microarray probe level data
Support for development of our software and tools to increase their usefulness to the research community.

U41HG007000 (Weng) 09/21/12 – 07/31/17
NIH/NHGRI (Subcontract: UMass Medical Center)
EDAC: ENCODE Data Analysis Center
In this project, Dr. Irizarry will provide advice on experimental design, quality management, and data analysis.
Role: Co-Investigator

U41HG007000 (Weng) 09/28/16 – 07/31/17
NIH/NHGRI (Subcontract: UMass Medical Center)
EDAC: ENCODE Data Analysis Center
This supplement supports the ENCODE Data Analysis Center (EDAC), consisting of a multi-disciplinary group of leading scientists who respond to directions from the Analysis Working Group (AWG) of ENCODE and thus integrate data generated by all groups in the ENCODE Consortium in an unbiased manner. These analyses substantially augment the value of the ENCODE data by integrating diverse data types. Role: Co-Investigator

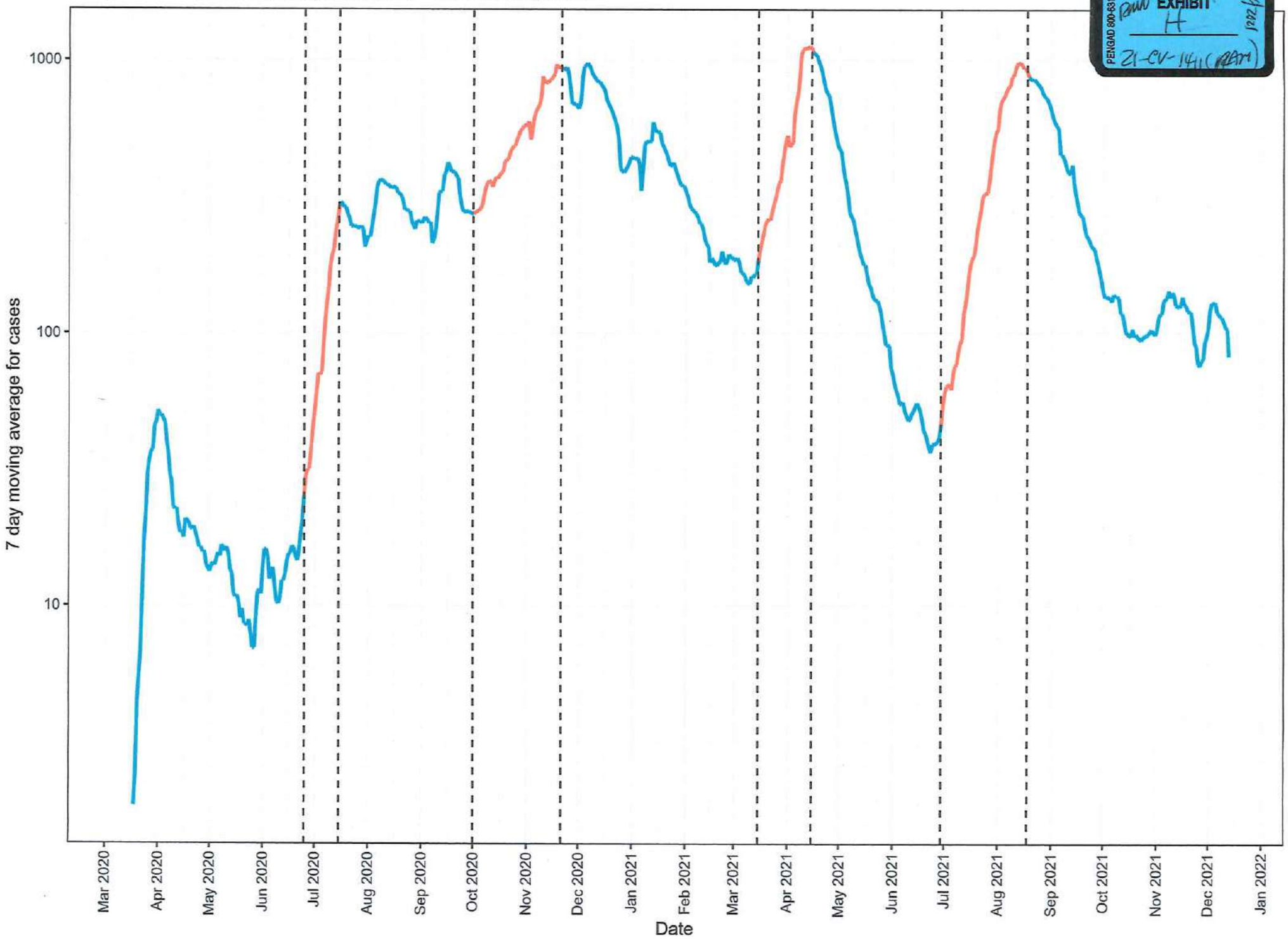
R21CA185787 (Tyekucheva) 12/23/14 – 11/30/16
NIH/NCI
Statistical Methods for Transcriptome Profiling from Archival Tumor Samples
This proposal is to free open source FFPE-specific analytic tools, validate them theoretically and empirically, and use them to investigate prostate cancer molecular subtypes in a large and well-annotated cohort.
Role: Co-Investigator

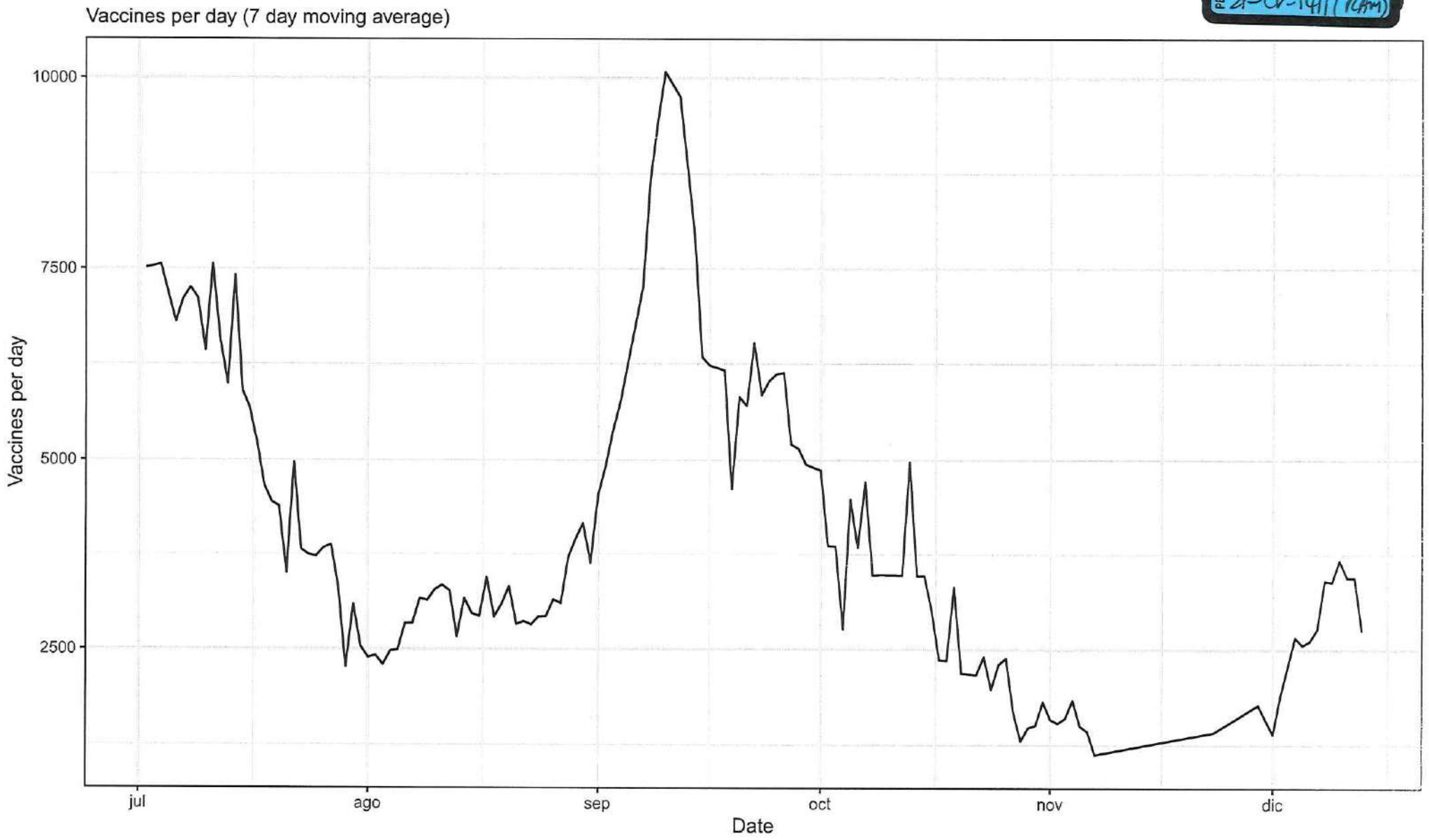




Detected cases: 7 day moving average in log scale

In log−scale linear trend (highlighted in red) = exponential growth.





CERIFIED TRANSLATION

GOVERNMENT OF PUERTO RICO
LA FORTALEZA
SAN JUAN, PUERTO RICO

Administrative Bulletin Number: OE-2021-075

EXECUTIVE ORDER OF THE GOVERNOR OF PUERTO RICO, HON. PEDRO R. PIERLUISI, TO IMPLEMENT VARIOUS INITIATIVES AGAINST COVID-19, AND TO REPEAL ADMINISTRATIVE BULLETINS NOS. OE-2021-058, OE-2021-062, OE-2021-063, AND OE-2021-064.

WHEREAS: Since March 12, 2020—after the first cases of the disease known as COVID-19, which is caused by the new SARS-CoV2 coronavirus, were reported on the island— we have been in a state of emergency. From said date on, countless strategies have been implemented to control the pandemic, including the mask and social distancing mandates. The last measure was the promulgation of administrative bulletins nos. OE-2021-058, OE-2021-062, OE-2021-063, and OE-2021-064, which required the members of certain important sectors of the society to be vaccinated against said virus, subject to certain exceptions and alternatives available.

WHEREAS: After said mandates, we have experienced a decrease in infections in recent months. Particularly, the daily average of confirmed cases is at 63 positive cases. When the vaccination mandates were being first promulgated, such statistic was at 233. Likewise, hospitalizations are at a total of 52 adults and 3 children. This represents 1% of the total beds available. By contrast, in August, adult hospitalizations increased by 7%. As to intensive care units, there are 9 adults and 1 child hospitalized as of today. Statistically this represents 1% for adults as well as children. In August, these statistics were around 21% in adults and 5% in children. The positivity rate, that is, the percentage of people who test positive to the virus out of all the people who get tested, is at an average of 3.1%, which constitute a significant decrease compared to August, when it reached 11.27%.

The foregoing notwithstanding, the deaths have also dropped significantly. By late August, we experienced a daily death average of around 14.3 cases daily. Today, this statistic is at 1 case daily.

I, Juan E. Segarra, USCCI #06-067/translator, certify that the foregoing is a true and accurate translation, to the best of my abilities, of the document in Spanish which I have seen.

WHEREAS: Vaccination has been an important factor in this improvement. Proof of this was that while the aforementioned statistics were decreasing, Puerto Rico was standing out as the jurisdiction with the highest number of persons vaccinated against COVID-19 in the United States and the Americas. According to the CDC's data, over 93.2% of persons older than age 12 have at least one doses. Moreover, 74.2% of the total population of the island is duly vaccinated; thus, we are the jurisdiction with the highest percentage of the population fully vaccinated in the United States.

WHEREAS: The World Health Organization (WHO) has provided that the vaccines available are safe and efficient, and that they prevent people from falling seriously ill or dying as a result of being infected with SARS-CoV2. In turn, vaccination reduces the likelihood of infecting other persons. Hence, WHO is encouraging vaccination, even for people who have been infected with COVID-19.

Likewise, the United States Food and Drug Administration (FDA) has issued emergency use authorizations for three (3) COVID-19 vaccines and has stated that they actually work by preventing the disease and its serious health consequences including hospitalization and death. In turn, it stated that the available information suggests that the authorized vaccines protect against strains or variants currently spreading. Thus, the FDA —the agency concerned with evaluating and authorizing vaccines— has promoted immunization as an effective tool to reduce COVID-19 spreading.

Lastly, the Centers for Disease Control (CDC) have stated that COV1D-19 vaccines are safe and effective, especially in preventing COVID-19 and its potential serious complications, such as death. Vaccines are even effective against the known variants. They asserted that vaccines can prevent people from getting infected or spreading the virus. They particularly provided that COVID-19 vaccines protect people from the symptoms, but also help people from getting infected with the virus that causes COVID-19. Vaccination may prevent the spreading of this disease, but also

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helps protect people and those around them. Although they recognize that there is a possibility of vaccine breakthrough infections – given that no vaccine is 100% effective – they prevent people from getting seriously ill and help protect their families and people around them. In turn, the CDC clarified these vaccines are not experimental, for they have already undergone the clinical trial stages. Even one of the vaccines already has been duly approved for a certain sector of the population. Hence, the CDC asserts that the most effective option to combat the pandemic is to be vaccinated against COVID-19.

WHEREAS: The scientific data in Puerto Rico shows the great effectiveness the vaccine has had. Particularly, it was concluded that the risk of infection for unvaccinated individuals is 6.9 times more than for vaccinated individuals. As to hospitalizations, unvaccinated individuals are 12.2 times more likely to be hospitalized than vaccinated individuals. Lastly, as to deaths, the risk of death for unvaccinated individuals is 25.7 times more than for vaccinated individuals. That is, vaccination is at least 3 times better to prevent infection, 8 times better to prevent hospitalizations, and 16 times better to prevent deaths associated with COVID-19.

WHEREAS: The data issued by the CDC is equally surprising. According to the studies, unvaccinated individuals are 6.1 times more at risk of testing positive to COVID-19 and 11.3 times more at risk of dying as a result of COVID-19, vis à vis vaccinated individuals. In the case of mRNA vaccines, it was concluded that in both studies under real conditions and clinical trials, they offered equal protection by reducing the risk of being infected or seriously ill by 90% or more in fully vaccinated individuals.

WHEREAS: Despite how successful the vaccination process has been, there is still a significant number of unvaccinated individuals, even when unvaccinated individuals are at a serious risk of getting infected or spreading COVID-19. This situation seriously affects other people. Scientific studies even explain that unvaccinated individuals as well as the spreading of this disease can cause the appropriate environment for the development of new variants

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that could be just as bad or worse than the Delta variant. Moreover, each unvaccinated individual are at risk of getting seriously ill, hospitalized, ending up in the intensive care unit, dying or developing a long-term health complication –known as long COVID-19– such as respiratory failure, blood clotting, multi-organ effects, namely cardiovascular, neurologic, or neurocognitive effects, damages to the gastrointestinal system and other organs, general wellbeing deficiencies, including discomfort, fatigue, musculoskeletal pain, and diminished quality of life, among other permanent and incapacitating health effects.

WHEREAS: According to scientific studies, unvaccinated individuals – including asymptomatic and presymptomatic individuals – are the ones contributing most significantly to the community spread of SARS-CoV-2. Unvaccinated individuals are most likely to get infected and spread the virus to those around them. Moreover, the Delta variant has increased the contagiousness, especially between unvaccinated individuals, which has increased the risk of infection among vaccinated individuals given the lack of other mitigation strategies. This has occurred in different workplace scenarios.

Even if the Delta variant has decreased the effectiveness of the vaccine, the advantages thereof are undisputable. The medical evidence shows that vaccinated individuals infected with the Delta variant could spread the disease. Likewise, it has been found that both persons bear the same viral load. However, the infection and transmission in unvaccinated individuals is higher. That is, despite the viral load, unvaccinated individuals have a higher risk of transmission than vaccinated individuals, given that they are more likely to get infected with COVID-19. Moreover, the viral load in unvaccinated persons could disappear faster, hence, the infection period is shorter and the likelihood of transmission is lower.

WHEREAS: In view of this scenario, where there are still unvaccinated individuals, the CDC recommends performing a screening test. Contrary to the diagnostic test, (which are used to identify current COVID-19 infections), screening tests seek to identify asymptomatic infected persons who have not known, presumed, nor notified SARS-CoV-2 exposure. Screening tests help to

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identify unknown cases in order to take measures to prevent the subsequent transmission or fast spreading of COVID-19. The CDC recommends to perform these screenings to unvaccinated individuals at workplaces, as well as students, teaching staff, and employees of schools and higher education institutions, all of which are unvaccinated. In turn, the CDC recommends not performing the screening test on fully vaccinated individuals who have no symptoms and have not been exposed to COVID-19. That is, the test is recommended only when the vaccinated individual shows symptoms or has been closely in contact with a person who has tested positive to COVID-19.

Moreover, the CDC has indicated that screening tests should be performed in unvaccinated individuals in large workplaces who are more at risk of virus introduction (for instance, people who work with customers, such as restaurants and beauty salons) or who have a higher risk of transmission, such as places where it is difficult to observe social distancing.

The CDC's recommendation is to perform weekly screening tests on unvaccinated individuals. In doing so, employees infected with SARS-CoV-2 would be identified, thus, it would help prevent or reduce subsequent transmission, which constitutes an extremely important occupational health measure at the mentioned places. According to the CDC, outbreak prevention and control depends largely on frequently testing unvaccinated individuals.

Consequently, in view of this circumstances, it is necessary to promote weekly testing as well as the simplest, most effective and efficient measure against this disease: vaccination. In doing so, we also protect other persons.

WHEREAS: Regarding precautionary measures to avoid infection, even if scientific studies recognize their importance in the efforts of preventing virus exposure, it has been stated that they are focused on prevention rather than the immune system of individuals so as to address potential exposure. Hence, the effectiveness thereof depends on the individual responsibility of each person and the effectiveness of the personal protective equipment they use. Particularly, there is a risk associated to a human error when the

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appropriate social distancing is not observed, a positive case is not properly reported, and when the protective equipment was not properly used or was not properly cleaned and stored after each use, and are not replaced when they lose effectiveness. On the contrary, the vaccine works automatically with long-term effects given that it works with the immune system and does not rely on any human effort whatsoever. Hence, no other factors must be relied on, namely, equipment efficiency or the actions of other persons. In this manner, the vaccine is the most efficient and effective tool of our society.

WHEREAS: As to the validity of vaccination, as stated in Administrative Bulletin No. OE-2021-058, the Supreme Court of the United States has examined the power of the State to regulate the use thereof. See, *Jacobson v. Massachusetts*, 197 U.S. 11 (1905) and *Zucht v. King*, 260 U.S. 174 (1922). In both cases, the Supreme Court of the United States validated the state's authority to reasonably mandate vaccination.

WHEREAS: As a result of said decisions, vaccination has been considered throughout history as a critical tool to achieve the health and security objectives, particularly of addressing infectious and highly contagious diseases. So much so that various vaccines are mandatory since the 19th century, which has led to control various diseases. In the United States, mandatory vaccination include: diphtheria, tetanus, pertussis, polio, measles, rubella, chickenpox, and mumps, among others. In Puerto Rico, mandatory vaccination includes also diphtheria, tetanus, pertussis, hepatitis B, measles, rubella, chickenpox, and mumps, among other diseases. Therefore, the mandatory vaccination is not new and have been an additional tool for years to safeguard the health of the population.

WHEREAS: In Puerto Rico, in *Lozada Tirado v. Testigos de Jehova*, 177 DPR 893 (2010), our Supreme Court recognized that, even though the people have a right to reject medical treatment, this is not an absolute right. The Court concluded that there may be certain interests of the State that may be taken into account, such as the protection of innocent third parties. In doing so, it recognized that the State may establish certain vaccines as mandatory requirements when there is a threat of an epidemic. *Id.*, n. 13.

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WHEREAS: In the case of COVID-19 vaccines, despite the lack of pertinent case law, most challenges to the vaccination mandates have not prevailed. Particularly, the Supreme Court of the United States has opted to reject cases challenging certain vaccination mandated. As recently as October 29, 2021, said Court rejected a stay in a vaccination mandate issued by the Government of Maine to health employees, even when it did not recognize a religious belief exception. See, *Does 1-3 v. Mills*, No. 21A90, 595 U.S. ____ (2021). In said case, the U. S. Court of Appeals for the First Circuit upheld the vaccination mandate of the Government of Maine. See, *Does 1-3 v. Mills*, No. 21-1826. Moreover, the U. S. Supreme Court also refuse to review a determination of the U. S. Court of Appeals for the Seventh Circuit which validated the vaccination mandate for students of the University of Indiana. See, *Klaasen et al v. The Trustees of Indiana,* 7 F. 4th 592 (2021).

Likewise, on October 29, 2021, the U. S. Court of Appeals for the Second Circuit upheld the vaccination mandate of New York. See, We *The Patriots USA Inc. et al. v. Hochul*, et al. No 21-2179, and *Dr. A. v. Hochul*, No. 21-2566.

Furthermore, in *Bridges v. Houston Methodist Hospital*, 2021 WL 2399994, a federal district court upheld a Houston's hospital vaccination mandate for employees. It held that vaccination as a condition for employment is not coercion and is valid as such.

WHEREAS: In Puerto Rico, the courts have had substantial cases under their consideration recently where vaccination mandates have been validated. The first was before the Court of First Instance, *Lourdes Amadeo Ocasio, et al. v. Pierluisi et al.* SJ2021CV04779, which upheld the vaccination mandate at the schools of Puerto Rico and provided that "the State as a compelling interest to safeguard the public health and take all measures as are necessary to effectively combat a pandemic that has affected the life of everyone in this planet and simply has no precedents in our modern history. Undoubtedly, these measures include requiring vaccination against said disease as well as masking in places that promote gatherings indoors, such as schools and universities." In turn, it concluded that

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"because we understand that the executive and administrative orders in controversy are based on reliable and proven scientific data, and that because they are carefully designed to allow reasonable accommodation to persons who are eligible and avail themselves thereof, we conclude that they are valid and fully adjust to the applicable constitutional parameters."

Moreover, on November 1, 2021, the U. S. District Court for the District of Puerto Rico rendered a judgment dismissing a complaint filed by several public employees challenging Administrative Bulletin No. OE-2021-058. The Court concluded that the measures taken serve a convincing public interest, are related to a health crisis, include reasonable options, and do not violate the due process of law or other legal provisions. See, *Rodríguez Vélez v. Pierluisi*, No. 21-1366 (PAD).

WHEREAS: On September 9, 2021the President of the United States, Joseph R. Biden Jr., signed two executive orders requiring all federal employees and contractors to be vaccinated or get tested for COVID-19 on a weekly basis. In turn, on September 24, 2021, the Safer Federal Workforce Task Force issued guidelines for contractors and subcontractors of the Federal Government.

Moreover, on November 4, 2021, the Occupational Safety and Health Administration (OSHA) of the U. S. Department of Labor and Human Resources issued an Emergency Temporary Standard (ETS) requiring employers having 100 employees or more to ensure that they are vaccinated or furnish a negative COVID-19 test result. This agency had previously issued another ETS imposing workplace safety requirements that are more stringent for workers rendering healthcare and medical support services. This rule currently applies to Puerto Rico. Likewise, the Centers for Medicare & Medicaid Services (CMS) announced a vaccination mandate for all healthcare employees of participating Medicare and Medicaid facilities.

In addition, various states and cities have implemented measures to require vaccination to employees and other sectors of the society.

WHEREAS: It should be noted that COVID-19 vaccination mandates in the United States and Puerto Rico have been effective, given that many people who

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were at a crossroads of whether to be vaccinated or furnishing a negative test result have opted to get vaccinated voluntarily.

WHEREAS: On November 2, 2021, the CDC approved the COVID-19 vaccine for children and teenagers ages 5 to 11. According to said entity, even though children have a lower risk of being seriously ill because of COVID-19 compared to adults, they could get infected with the virus that causes COVID-19, they could be seriously ill, suffer short- and long-term complications, and spread COVID-19 to others. In this sense, children infected could suffer serious complications such as the multisystem inflammatory syndrome (MIS-C) which causes inflammation in various parts of the body, such as the heart, the lungs, kidneys, brain, skin, eyes, or other gastrointestinal system organs. Moreover, the CDC indicates that children who are vaccinated help protect other family members, namely, siblings who are not eligible to be vaccinated. In addition, it allows for this population to remain at school and be able to participate in sporting activities, games, and other group activities safely.

As reported by the FDA, vaccine safety was studied in around 3,100 children ages 5 to 11 who received the vaccine and no serious side effects have been identified. Furthermore, the effectiveness found was 90.7%.

WHEREAS: Article 5.10 of Act No. 20-2017, as amended, better known as the "Puerto Rico Public Safety Department Act," empowers the Governor to declare a state of emergency on our Island, and subsequently enact any measures as necessary for the duration of the emergency to manage it in order to protect the safety, health, and property of all the residents of Puerto Rico.

WHEREAS: Subsection (b) of Section 5.10 of Act No. 20-2017, provides that the Governor of Puerto Rico may prescribe, amend, and revoke any regulations as well as issue, amend, and rescind such orders as deemed convenient which shall be in effect for the duration of the state of emergency or disaster. Regulations prescribed or orders issued during a state of emergency or disaster shall have force of law for the duration of the state of emergency or disaster.

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WHEREAS: Section 1.018 of Act No. 107-2020, as amended, known as the "Puerto Rico Municipal Code," provides that when the Governor of Puerto Rico declares a state of emergency, the mayors shall be relieved from having to issue an executive order for the same purposes, and that the Governor's executive order shall prevail and have full effectiveness as if it were promulgated by the Mayors.

WHEREAS: The power to govern a people entails a great responsibility of ensuring that the population is safe and secure. In turn, the State's police power—as delegated to the Executive Power under Act No. 20-2017—empowers the government to take measures as are necessary to protect the health and safety of its population. In other words, it is the inherent power of the State that allows it to create and promote regulation in general in order to protect the health, safety, and general welfare. In order to achieve these benefits in favor of the community, the State has the power to restrict certain personal interests, which are not absolute.

WHEREAS: Given that the pandemic has proven that cases tend to arise in waves, a rise is possible at any given time more so when we have mostly returned to normal and there are indoor spaces, such as workplaces, where there are multiple persons in contact for extended periods of time, there is little ventilation, they spend too much time near one another and share common areas such as bathrooms, meeting centers, or lunch rooms. Hence, in order to avoid significant increases, it is necessary to maintain certain measures and include other definite actions within the more vulnerable sectors. Vaccination is without a doubt the most important measure to reduce the risk of anyone falling seriously ill, being hospitalized, or even dying.

As a result of this, it is necessary to promote vaccination among the various sectors.

One of the main sectors is our children given that we have to ensure their health so as to allow them to continue attending school in person, which they have missed dearly. The CDC has promoted vaccination at schools for it helps to return to schools as well as out of school and sporting activities safely. It indicates having all eligible students as well as teachers

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and other personnel as well as members of the family unit vaccinated against COVID-19 is the most critical strategy to help schools fully resume their activities safely.

Secondly, it is necessary to reinforce our health system, hence, the employees of this sector must be vaccinated save for the limited constitutional exceptions. The grounds therefor are that the CDC has recognized that the medical care personnel continues to be the first line of defense against COVID-19. They render essential services to persons who are or could be infected with the virus that causes COVID-19, thus, medical care personnel have a higher risk of being exposed to or infected with the virus. For such reason, the CDC has stated that all medical care personnel must be vaccinated against COVID-19.

Lastly, there are workplaces where there is a significant number of persons which poses an actual risk of infection. Note that the CDC has identified workplaces where employees remain for an extended period of time, between eight (8) and twelve (12) hours per shift, or where employees are in near and prolonged contact with their colleagues as high-risk. In turn, it has recommended to perform screening tests in large workplaces. Therefore, it is necessary to require vaccination or a weekly COVID-19 test result in medium- and large-sized companies. In Puerto Rico, pursuant to the rules of the Department of the Treasury, a business is considered to be medium-sized when it has fifty (50) or more employees. Certainly, a gathering of fifty (50) persons or more who are in constant contact during work shifts poses a significant risk to everyone. Moreover, consistent with the statistics of the Department of the Treasury, in Puerto Rico we have over 4,700 employers with over fifty (50) employees which are considered medium-sized businesses, a number that is double the number of employers with one hundred (100) employees. Thus, given the composition of our economic sector, it is necessary to implement the recommendations of the CDC for employers with fifty (50) or more employees, which shall be effective and allow for the prevention of future infections.

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WHEREAS: I, PEDRO R. PIERLUISI, Governor of Puerto Rico, by virtue of the powers inherent to my office and the authority vested in me by the Constitution and the Laws of the Government of Puerto Rico, hereby declare and order the following:

Section 1: **PUBLIC POLICY.** This Executive Order has the purpose of compiling all the provisions in effect to address the COVID-19 emergency. The main purpose thereof is to establish measures as necessary to preserve the life of all the population of Puerto Rico, thus preventing the spreading and transmission of the virus that causes COVID-19 on our Island, including the new variants thereof. Increasing the rate of vaccinated individuals is critical to fight the pandemic. Particularly, this order is promulgated to address specifically the sectors of education, health, and employers with a high volume of employees. Therefore, this executive Order shall be interpreted and implemented so as to achieve said objectives.

Section 2: **QUARANTINE ORDER**. Under the authority granted by the Constitution of Puerto Rico, by Act No. 20-2017, as amended, and by Act No. 81 of March 14, 1912, as amended, I hereby order any person who is not fully vaccinated and is reasonably suspected to have been exposed to COVID-19, regardless of whether or not he shows signs of infection, to remain in quarantine for a period of fourteen (14) days. The Department of Health may reduce such quarantine period to ten (10) days without the person having to be submitted to a molecular test, or to seven (7) days if the person shows a negative result upon being submitted to a molecular COVID-19 test within five (5) days after being exposed to the virus. Likewise, the Department of Health is empowered to establish quarantine periods for other groups as he believes is in the interest of safeguarding public safety.

For purposes of this Executive Order, a person is deemed to be fully vaccinated against COVID-19 two (2) weeks or more after having received the second dose of a two-dose series, or two (2) weeks or more after having received the one-dose vaccine, as approved or authorized by the FDA or any other included in the emergency use list of the WHO.

The purpose of the quarantine is to maintain a person that could have been

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exposed to the virus isolated from other persons in order to prevent or limit infection and spreading of the virus known as COVID-19. A quarantine entails that the person must remain in his home and physically distant from others. Said person must restrict his movements outside of the residence so as to avoid the risk of infection within the community. Moreover, every citizen who has been in contact with some who has tested positive to COVID-19 is hereby instructed to get tested through a molecular or viral test available within the fifth (5th) and seventh (7th) day after the last exposure. Failure to meet this requirement of remaining quarantined, as provided in this section, shall be deemed to be a violation of this Executive Order.

Except if the Department of Health issues any communication or guideline on the contrary for any interest group, the following persons shall not be required to remain quarantined or to get tested through a diagnostic test upon being exposed to COVID-19: 1) asymptomatic persons who are fully vaccinated; 2) persons who have had a positive COVID-19 diagnostic test in the last three (3) months after being exposed to the virus and having recovered.

Section 3: **ISOLATION ORDER.** Under the authority granted by the Constitution of Puerto Rico, by Act No. 20-2017, as amended, and by Act No. 81 of March 14, 1912, as amended, I hereby order any person who has been infected with the virus to be physically isolated for at least ten (10) days counted from the beginning of the symptoms, and with the potential to be extended contingent upon the investigation to be conducted on COVID-19 cases. The purpose of the isolation is to maintain infected persons away from the rest of the population, even at their homes. This means that the person must be confined and restrict his movements to avoid posing a threat to public health and prevent transmission to other persons. A person infected with COVID-19 who shows no symptoms or minor or moderate symptoms may end the isolation period when the following three (3) criteria are met:

1) at least ten (10) days since the beginning of the symptoms have elapsed (or since the first sample was taken and upon a subsequent positive test result, for asymptomatic individuals);

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2) there was no fever (without using anti-fever medications) within the last twenty-four hours; and

3) shows an improvement in other symptoms associated with COVID-19.

Persons who test positive to COVID-19 do not have to wait for a negative test result to conclude their isolation period if they meet the criteria established in the Case Investigation and Contact Tracing of the Department of Health. Patients who tested positive to COVID-19 and violate this isolation order thus posing a risk to others, shall be subject to criminal liability under Act No. 146-2012, as amended, known as the "Puerto Rico Penal Code," in addition to the sanctions for noncompliance with this Executive Order.

Section 4: **INDIVIDUAL PRECAUTIONARY MEASURES.** Any person who is in contact with any other person outside of his family unit shall comply with the following protective measures:

1. Cover his mouth and nose with a mask or scarf made of fabric or other material pursuant to the instructions of the Department of Health and the following guidelines:

a. Every person shall use a face mask at indoor places, such as businesses, medical offices, casinos, places that offer financial, consumer, professional, nonprofessional, college and postsecondary, or religious services, movie theaters, stadiums, bars, among others, regardless of his COVID-19 vaccine status. Persons who participate in gatherings of twenty (20) persons or less where all the attendees are fully vaccinated, or where masking is inconsistent with or affects their health shall be exempt.

b. Masking shall be required in outdoor spaces, even when the person is partially or fully vaccinated, when the activity scheduled or organized entails the gathering of fifty (50) or more persons. Masking is recommended in tourist places where visitors are also gathered.

c. Hospital and healthcare center employees shall use KN-95 or N-95 masks.

d. The Department of Health may require the use of face masks in other settings, as it determines to prevent future infections.

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e. A "mask" shall be understood to be any product made of fabric or other material to cover the mouth, nose, and chin that has a head harness and may surround the head or be held behind the ears. The foregoing shall comply with the recommendations and specifications of the Department of Health and the CDC.

2. Maintain a minimum of six (6) feet between persons other than the members of his family unit, thus avoiding gatherings.

3. Wash his hands regularly with soap and water, or with disinfectants approved by the health officials.

Section 5: **MASS GATHERINGS.** In order to be able to safeguard the health of the people of Puerto Rico and reduce infections, I hereby order that as of the effectiveness of this Executive Order all indoor establishments that carry out mass gatherings, that is, theaters, stadiums, convention and activity centers, and any other place where activities that promote the gathering of persons take place shall abide by the following rules:

1. The organizers, owners, administrators, or similar persons who carry out and organize events, or conduct public or private operations that promote the gathering of persons and wish to operate the venue at 100% capacity shall be compelled to require attendees to have received a full COVID-19 vaccine series approved by the FDA, or any other vaccines included in the WHO's emergency use list. The event's organizer shall be responsible for requesting attendees to show the COVID-19 Vaccination Record Card or Vacu-ID as proof of vaccination. Moreover, attendees shall be responsible for showing their COVID-19 Vaccination Record Card or Vacu-ID as proof of vaccination in order to attend the event in person.

2. As an alternative to the previous subsection, the organizers, owners, administrators, or similar persons who carry out and organize events, or conduct public or private operations that promote the gathering of persons may allow the participation of unvaccinated persons, provided that they show a negative COVID-19 test result from a qualified virus test (Nucleic Acid Amplification Test or NAAT and antigen tests) performed within a maximum of seventy-two (72) hours prior to arriving at the venue and which has been processed by an authorized health professional. Likewise,

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attendees shall also be allowed to furnish a positive COVID-19 test result performed within the last three (3) months, together with the pertinent documents of his recovery, including a letter from a certified healthcare provider or a government health official certifying that said person is recovered and ready to be at a public place. When the organizers, owners, administrators, or similar persons chose this alternative, the venue may operate at 50% capacity.

3. In view that the vaccination process for minors between the ages of five (5) and eleven (11) began recently, they may attend mass gatherings held at indoor places until January 31, 2022, by furnishing a negative COVID-19 test result from a qualified virus test (Nucleic Acid Amplification Test or NAAT and antigen tests) performed within a maximum of seventy-two (72) hours prior before arriving at the venue and which has been processed by an authorized health professional. Beginning on February 1, 2022, said minors shall be governed by the provisions of subsections 1 and 2 of this Section.

4. Given that vaccines are not authorized for children younger than five (5) years of age, as a general rule, shall not attend mass gatherings even with a test result from a qualified virus test. The Secretary of the Department of Health or his delegate shall have discretion to evaluate any petition for exemption for these minors to attend specific activities where their health is ensured.

5. The foregoing shall not apply to religious events or public events where government services are offered.

Moreover, I hereby order that as of the effectiveness of this Executive Order, every organizer, owner, administrator, or similar person of mass gathering in outdoor venues that promotes the gathering of five hundred (500) persons or more shall coordinate with the Department of Health to establish the protocol to be followed to ensure that the event is safe for the health of all attendees. This includes the masking requirement throughout the activity and determining whether children age five (5) or younger may attend.

At mass gatherings in outdoor venues that promote the gathering of five hundred (500) persons or more, attendees shall only be required to wear

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face masks at all times. In these events, however, the Department of Health shall be empowered to require any specific protocol when deemed necessary to ensure the health of all attendees.

In the case of recreational or sporting events, the Department of Sports and Recreation, in consultation with the Department of Health shall determine the appropriate protocol for each event, if any.

Section 6: **VACCINATION OR NEGATIVE COVID-19 TEST RESULT REQUIREMENT FOR GOVERNMENT EMPLOYEES AND CONTRACTORS.** In order to minimize infections and safeguard government services, I hereby order that as of the effectiveness of this Executive Order, the following provisions shall be complied with:

A. Government employees or anyone who works in person. Employees or contractors who work in person at the public agencies of the Executive Branch, in addition to contractors and their employees or frequently visit government offices, regardless of their duties, shall comply with the following conditions:

1. To furnish their employers proof of being fully vaccinated against COVID-19 with an FDA approved or authorized vaccine, or others included in the WHO's emergency use list;

2. To get tested, at their expense, every seven (7) days through a qualified SARS-CoV-2 virus test (Nucleic Acid Amplification Test or NAAT and antigen tests) approved by the FDA and processed by an authorized health professional, and furnish the negative result of said test at least every seven (7) days; or

3. To furnish their employers with a positive COVID-19 test result performed within the last three (3) months, together with the pertinent documents of the person's recovery, including a letter from a certified healthcare provider or a government health official certifying that said person is recovered and is ready to be at a public place.

It shall not be necessary for employees and contractors to furnish the documents associated with a medical or religious exception in order to comply with the second or third condition.

B. Municipal employees to which the mandate applies for the first

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time. In the case of municipal employees, they must comply with any of the three aforementioned conditions. However, given that it is the first time that this mandate applies to them, in order to comply with the first condition, it shall be sufficient for these employees to furnish within fifteen (15) days from the effectiveness of this Executive Order, proof of having begun their vaccination process by receiving the first dose. These employees, however, must comply with and furnish subsequent proof to their employers of having received the second dose, if the vaccine series so requires it, within forty-five days from the effectiveness of this Executive Order.

C. Responsibilities. Every employer –or his delegate– shall be responsible for requiring a person or employee to furnish the COVID-19 Vaccination Record Card or Vacu-ID or document attesting to having completed or begun the COVID-19 vaccination process, as the case may be, or a negative COVID-19 test result from a qualified SARS-CoV-2 virus test (Nucleic Acid Amplification Test or NAAT and antigen tests) or a positive COVID-19 test result performed within the last three (3) months, together with the pertinent documents of the person's recovery. The vaccination record may be furnished in another authorized physical or digital format to certify vaccination.

In the case of contractors of the Executive Branch, they shall be responsible for ensuring that their employees comply with the provisions herein and notify the contracting agency of their compliance with this Executive Order.

D. Noncompliance. Failure to comply with the provisions of this section by the aforementioned persons shall entail the following measures:

a. Government employees – including the employees from the Executive Branch and the Municipalities – may not work in person. Hence, the employer shall take the applicable measures as pertinent, including allowing them to avail themselves of compensatory time, applicable regular leaves, or a leave of absence, as applicable.

b. In the case of a government contractor or his employees, these may not visit the government agency and the latter may take measures as pertinent regarding the contract executed therewith, which may include,

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but not be limited to the termination of said contract.

E. Definition. For the purposes of this Executive Order, the term "employee" shall be interpreted broadly and includes any natural person who works in person or teleworks for wages, or a salary, compensation, emoluments, or any type of remuneration. For the purposes of the vaccination requirement, as established in this Executive Order, any person who renders voluntary services at these places shall also be deemed to be employees.

Section 7: **VACCINATION REQUIREMENT FOR EMPLOYEES OF HEALTH SECTOR.** Regardless of the guidelines issued by the CMS, and in order to avoid complications in the health system and guarantee the operations thereof, I hereby order that, as of the effective date of this Executive Order, all employees or persons who work at healthcare facilities, regardless of their duties, shall be fully vaccinated against COVID-19 with a vaccine approved by the FDA, or any of the other vaccines included in the WHO's emergency use list. The foregoing shall be subject to any applicable medical or religious exception, as explained in this section, then, the employee shall get tested, at his expense, every seven (7) days through a qualified SARS-CoV-2 virus test (Nucleic Acid Amplification Test or NAAT and antigen tests) approved by the FDA and processed by an authorized health professional, and furnish the negative result of said test at least every seven (7) days; or furnish a positive COVID-19 test result performed within the last three (3) months, together with the pertinent documents of the person's recovery, including a letter from a certified healthcare provider or a government health official certifying that said person is recovered and is ready to be at a public place. It shall be understood that for an employee of the health sector the virus test or the positive result option shall be available when they furnish proof of a medical or religious exception.

Every employer –or his delegate– shall be responsible for requiring a person or employee to furnish the COVID-19 Vaccination Record Card or Vacu-ID or document attesting to having completed or begun the COVID-19 vaccination process, as the case may be, or as an exception

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a negative COVID-19 test result from a qualified SARS-CoV-2 virus test (Nucleic Acid Amplification Test or NAAT and antigen tests) or a positive COVID-19 test result performed within the last three (3) months, together with the pertinent documents of the person's recovery. Furthermore, the person or employee shall be responsible for furnishing the COVID-19 Vaccination Record Card or Vacu-ID or document attesting to having completed or begun the COVID-19 vaccination process, as the case may be, or as an exception a negative COVID-19 test result from a qualified SARS-CoV-2 virus test (Nucleic Acid Amplification Test or NAAT and antigen tests) or a positive COVID-19 test result performed within the last three (3) months, together with the pertinent documents of the person's recovery. The vaccination record may be furnished in another authorized physical or digital format to certify vaccination.

For a medical exception to apply, the employee shall prove that his immune system is compromised, he is allergic to the vaccines, or has any other medical contraindication that prevents him from getting vaccinated. This shall be certified by a physician authorized to practice in Puerto Rico. The physician shall also certify the duration of the medical contraindication and whether it is temporary or permanent. In the event that it is temporary, once the contraindication ceases, the person shall fulfill the vaccination mandate established in this Order, as applicable.

Moreover, in the event of an exception on the basis of religious beliefs, the person must furnish an affidavit of religious objection whereby such person – together with his minister or spiritual leader, or by himself– states that on the basis of his sincerely-held religious beliefs, he cannot receive a COVID-19 vaccine. It shall state specifically the nature of his refusal, an explanation as to how fulfilling the vaccination requirement imposes a substantial burden or is in conflict with his sincerely-held religious beliefs, practice, or observance; the time during which said person has observed or practiced said religious beliefs; the type of vaccine refused and if such person has been vaccinated recently. This religious exception does not protect a person based on social, political, economic, or personal

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preferences. The employer shall assume that the religious exception is based on sincerely-held religious beliefs. However, he is empowered to require more information to ensure that said beliefs are sincerely-held. The employer, however, may not question the reasonableness of said religious belief.

Noncompliance with the foregoing by the aforementioned persons shall prevent them from working in person. Hence, the employer may take the applicable measures as pertinent, including allowing said employee to avail himself of compensatory time, applicable regular leaves, or a leave of absence, as the case may be.

For purposes of this Executive Order, "healthcare facilities" mean places where direct healthcare services are rendered to the population. Particularly, these include, but are not limited to hospitals, clinical laboratories, emergency rooms, medical service clinics, health centers, primary care physicians' and specialists' offices, therapy centers, blood banks, pharmacies, all older adult care center, and cannabis dispensaries, among others.

Section 8: **VACCINATION REQUIREMENT FOR THE EDUCATION SECTOR.** To avoid affecting the education system and thus ensure the continuation of services, I hereby order that as of the effectiveness of this Executive Order, the following shall be complied with:

A. Students age twelve (12) or older. By virtue of the powers granted by Act No. 81 of March 12, 1912, as amended, and Act No. 25 of September 25, 1983, as amended, I hereby order that students age twelve (12) or older – including college students and students of technical education institutions – of any private or public entity shall be fully vaccinated against COVID-19 in order to attend classes in person, subject to any applicable medical or religious exception, as explained in this section. In the case of an applicable exception, the student shall have two options: 1) get tested, at their expense, every seven (7) days through a qualified SARS-CoV-2 virus test (Nucleic Acid Amplification Test or NAAT and antigen tests) approved by the FDA and processed by an authorized health professional, and furnish the negative result of said test at least

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every seven (7) days to the director or his delegate; or furnish a positive COVID-19 test result performed within the last three (3) months, together with the pertinent documents of the person's recovery, including a letter from a certified healthcare provider or a government health official certifying that said person is recovered and is ready to be at a public place; or 2) receive online education – if available – or other alternative education.

B. Students ages five (5) to eleven (11). Given that vaccination against COVID-19 was recently approved for children ages five (5) to eleven (11), I hereby order that they shall be fully vaccinated against COVID-19 by January 31, 2022, in order to attend school in person at the public or private educational institutions. These students shall be subject to any applicable medical or religious exceptions, as explained in this section. If an exception is granted, children ages five (5) to eleven (11) shall not have to furnish a weekly COVID-19 test result. However, random test may be conducted to detect any potential COVID-19 infection. If not eligible for any of the exceptions, the student shall receive online education – if available – or other alternative education.

In those cases where the student turns five (5) years-old after the effectiveness of this Executive Order, such student shall have until January 31, 2022 or sixty (60) days from his birth date, whichever is longer, to complete the vaccination process.

C. Teaching and non-teaching personnel and contractors. I hereby order teaching and non-teaching personnel and contractors of public or private schools, education centers and universities, to be fully vaccinated against COVID-19 in order to be able to offer services to the school community, subject to the applicable medical and religious exceptions, as explained in this section. In these last cases, the employee shall get tested, at his expense, every seven (7) days through a qualified SARS-CoV-2 virus test (Nucleic Acid Amplification Test or NAAT and antigen tests) approved by the FDA and processed by an authorized health professional, and furnish the negative result of said test at least every seven (7) days; or furnish a positive COVID-19 test result

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performed within the last three (3) months, together with the pertinent documents of the person's recovery, including a letter from a certified healthcare provider or a government health official certifying that said person is recovered and is ready to be at a public place. It shall be understood that for the teaching and non-teaching personnel, as well as contractors at schools, educational centers, and universities, the virus test or the positive result option shall be only available when they furnish proof of a medical or religious exception.

D. Responsibility. Every director of the educational centers or his delegate, together with the concerned employers –whether public or private– shall be responsible for requiring students, employees, or contractors to furnish the COVID-19 Vaccination Record Card or Vacu-ID or document attesting to having completed or begun the COVID-19 vaccination process, as the case may be, or a negative COVID-19 test result from a qualified SARS-CoV-2 virus test (Nucleic Acid Amplification Test or NAAT and antigen tests) or a positive COVID-19 test result performed within the last three (3) months, together with the pertinent documents of the person's recovery.

Moreover, the parents of underage students, students who are of legal age, employees, or contractors shall be responsible for furnishing the COVID-19 Vaccination Record Card or Vacu-ID or document attesting to having completed or begun the COVID-19 vaccination process, as the case may be, or a negative COVID-19 test result from a qualified SARS-CoV-2 virus test (Nucleic Acid Amplification Test or NAAT and antigen tests) or a positive COVID-19 test result performed within the last three (3) months, together with the pertinent documents of the person's recovery. The vaccination record may be furnished in another authorized physical or digital format to certify vaccination.

E. Applicable exceptions. For a medical exception to apply, the parents of underage students, students who are of legal age, employees, or contractors shall prove that his immune system is compromised, he is allergic to the vaccines, or has any other medical contraindication that

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prevents him from getting vaccinated. This shall be certified by a physician authorized to practice in Puerto Rico. The physician shall also certify the duration of the medical contraindication and whether it is temporary or permanent. In the event that it is temporary, once the contraindication ceases, the person shall fulfill the vaccination mandate established in this Order, as applicable.

Moreover, in the event of an exception on the basis of religious beliefs, the parents of underage students, students who are of legal age, employees, or contractors must furnish an affidavit of religious objection whereby such person – together with his minister or spiritual leader, or by himself– states that on the basis of his sincerely-held religious beliefs, he cannot receive a COVID-19 vaccine. It shall state specifically the nature of his refusal, an explanation as to how fulfilling the vaccination requirement imposes a substantial burden or is in conflict with his sincerely-held religious beliefs, practice, or observance; the time during which said person has observed or practiced said religious beliefs; the type of vaccine refused and if such person has been vaccinated recently. This religious exception does not protect a person based on social, political, economic, or personal preferences. The employer or the school shall assume that the religious exception is based on sincerely-held religious beliefs. However, said employer or school is empowered to require more information to ensure that said beliefs are sincerely-held. The employer or the school, however, may not question the reasonableness of said religious belief.

F. Noncompliance. Failure to comply with the provisions of this section by the aforementioned persons shall entail the following measures:

a. The student may not attend school in person. The student shall receive online education – if available – or other alternative education.

b. The teaching and non-teaching personnel may not work in person. Hence, the employer shall take the applicable measures as pertinent, including allowing them to avail themselves of compensatory time, applicable regular leaves, or a leave of absence, as applicable.

c. In the case of contractors, they may not work in person. In the case of a government contractor, the contracting agency may take

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measures as pertinent regarding the contract executed therewith, which may include, but not be limited to the termination of said contract.

Section 9: **VACCINATION REQUIREMENT FOR EMPLOYEES OF THE PRIVATE SECTOR.** In order to minimize contagion and safeguard the health of the people of Puerto Rico, I hereby order that as of the effectiveness of this Executive Order, the employees and persons working at hotels, *paradores*, lodgings, restaurants (including fast foods, food courts, and cafeterias) bars, "chinchorros," small cafeterias, sport bars, theaters, movie theaters, stadiums, convention and activity centers – whether indoor or outdoor – that sell alcoholic beverages or prepared food, beauty salons, barber shops, aesthetics salon, spas, gyms, child care centers (including Head Starts, and Early Head Starts) supermarkets, minimarts (including WIC authorized establishments), casinos, and convenience stores at gas stations – regardless of their function – shall comply with the following conditions:

1. to furnish proof of being fully vaccinated against COVID-19 with an FDA approved or authorized vaccine, or others included in the WHO's emergency use list;

2. to get tested, at their expense, every seven (7) days through a qualified SARS-CoV-2 virus test (Nucleic Acid Amplification Test or NAAT and antigen tests) approved by the FDA and processed by an authorized health professional, and furnish the negative result of said test at least every seven (7) days; or

3. To furnish a positive COVID-19 test result performed within the last three (3) months, together with the pertinent documents of the person's recovery, including a letter from a certified healthcare provider or a government health official certifying that said person is recovered and is ready to be at a public place.

Furthermore, I hereby order that in the case of employers that have fifty (50) or more employees, said employees shall comply with the aforementioned conditions. However, given that it is the first time that this mandate applies to them, in order to comply with the first condition, it shall be sufficient for these employees to furnish within fifteen (15) days from

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the effectiveness of this Executive Order, proof of having begun their vaccination process by receiving the first dose. These employees, however, must comply with and furnish subsequent proof to their employers of having received the second dose, if the vaccine series so requires it, within 45 days from the effectiveness of this Executive Order.

Employers with less than fifty (50) employees and that are not included in aforementioned list are encouraged to make adjustments as are necessary to require COVID-19 vaccination or weekly testing, even when they are not subject to the above requirements yet due to the economic implications it could have on their businesses vis à vis the benefits thereof.

Every employer, merchant, owner, manager, or similar person –or his delegate– shall be responsible for requiring a person or employee to furnish the COVID-19 Vaccination Record Card or Vacu-ID or document attesting to having completed or begun the COVID-19 vaccination process, as the case may be, or a negative COVID-19 test result from a qualified SARS-CoV-2 virus test (Nucleic Acid Amplification Test or NAAT and antigen tests) or a positive COVID-19 test result performed within the last three (3) months, together with the pertinent documents of the person's recovery. The vaccination record may be furnished in another authorized physical or digital format to certify vaccination.

Employers are encouraged to allow their employees to be vaccinated during working hours and grant them time as necessary to take care of any side effects, if any. To such effects, employees may use their accrued sick leave, if any. Employers, at their discretion, may also grant special vaccination leaves.

For the purposes of this Executive Order, the term "employee" shall be interpreted broadly and includes any natural person who works in person or teleworks – including the owner, merchant, manager, or similar person, as well as contractors, but not suppliers – for wages, or a salary, compensation, emoluments, or any type of remuneration. For the purposes of the vaccination requirement, as established in this Executive Order, any person who renders voluntary services at these places shall also be

deemed to be employees.

Section 10: **REQUIREMENT TO VISITORS.** In order to minimize contagion and safeguard the health of the people of Puerto Rico, I hereby order that as of the effectiveness of this Executive Order, restaurants (including fast foods, food courts, and cafeterias) bars, "chinchorros," small cafeterias, sport bars, theaters, movie theaters, stadiums, convention and activity centers that sell alcoholic beverages or prepared food, hotels, *paradores*, lodgings, beauty salons, barber shops, aesthetics salon, spas, gyms, and casinos, shall ascertain that all of their visitors – subject to the exceptions provided in this section – comply with one the following conditions:

1. to furnish proof of being fully vaccinated against COVID-19 with an FDA approved or authorized vaccine, or others included in the WHO's emergency use list;

2. to furnish a negative COVID-19 test result from a qualified SARS-CoV-2 virus test (Nucleic Acid Amplification Test or NAAT and antigen tests) performed within seventy-two (72) hours prior to his visit, and which has been processed by an authorized health professional; or

3. to furnish a positive COVID-19 test result performed within the last three (3) months, together with the pertinent documents of the person's recovery, including a letter from a certified healthcare provider or a government health official certifying that said person is recovered and is ready to be at a public place.

Every business or commercial establishment shall be responsible for requiring every visitor, as applicable – before entering the establishment – to furnish the COVID-19 Vaccination Record Card or Vacu-ID, or a negative COVID-19 test result from a qualified virus test, or a positive COVID-19 test result performed within the last three (3) months, together with the pertinent documents of the person's recovery. Also, the visitor shall be responsible for furnishing the COVID-19 Vaccination Record Card or Vacu-ID, or a negative COVID-19 test result from a qualified virus test, or a positive COVID-19 test result performed within

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the last three (3) months, together with the pertinent documents of the person's recovery. The vaccination record may be furnished in another authorized physical or digital format to certify vaccination.

It should be noted that the provisions of this Executive Order shall not limit the power of any private operator to implement restrictions in addition to the ones herein. That is, none of the provisions of this executive Order shall be construed as to preventing private operators from taking additional or more restrictive measures, including but not limited to any voluntary restriction to their business hours, self-limitation of space or number of persons who can be inside their businesses.

Children younger than age five (5) who cannot be vaccinated yet are exempt from the screening provided in this section. However, the provisions of this section shall apply to children age five (5) to eleven (11) – given that the vaccination process for them is under way – after January 31, 2022.

Likewise, in the case of restaurants (including fast foods, food courts, and cafeterias) bars, "chinchorros," small cafeterias, sport bars, any person who only and solely acquire food through delivery, curbside pickup, or pick up service, that is, who do eat inside the commercial establishment shall be exempt from this Section.

Any visitor who refuses to meet the requirements of this Executive Order, as implemented by the private operator, shall not enter the establishment. If the person is a guest at the hotel, *parador*, or lodging, including short-term rentals, such person may not visit nor stay at said place insofar as said person fails to comply with the provisions of this Executive Order. All citizens are encouraged to cooperate with private operators by complying with the provisions herein. In the event that a citizen fails to cooperate and attempts to force a private operator to incur in noncompliance with the provisions of this Executive Order, he may be subject to the provisions of Section 14 of this Order, and any other applicable provision of the Puerto Rico Penal Code.

Any restaurant (including fast foods, food courts, and cafeterias) bar, "chinchorro," small cafeteria, sport bar, theater, movie theater, stadium,

convention and activity center that sell alcoholic beverages or prepared food, hotel, *parador*, lodging, beauty salon, barber shop, aesthetics salon, spa, gym, or casino, that fails to meet the aforementioned requirements shall be required to limit the capacity of the business to 50%, in accordance with the Building Code in effect. (PR Building Code 2018).

Section 11: **OVERSIGHT.** The concerned agencies are hereby directed to oversee compliance with the provisions of this Executive Order. In turn, the public is encouraged to report to the pertinent authorities any entities that fail to comply with the provisions herein. In order to allow citizens to contribute to the oversight and full compliance with this Executive Order, every business or establishment is hereby ordered to have posters displayed in conspicuous places with the confidential COVID-19 hotline created by the Department of Health. The poster or advertisement shall state whether the establishment requires proof of vaccination or a negative COVID-19 test result to enter the premises. This sign or poster shall include the following contact information for citizens to report any noncompliance:

a) Telephone (787) – 522-6300, extensions 6899, 6840, 6824, 6833, 6893.

b) Email: investigaciones@salud.pr.gov

If said screening is not conducted at a business, said sign or poster shall include the number of persons that constitute the required 50% maximum occupancy, pursuant to the Building Code in effect (PR Building Code 2018), and as authorized by the Bureau of the Firefighters Corps of Puerto Rico; otherwise, it shall constitute noncompliance with this Executive Order.

Citizens are hereby encouraged to report to the concerned agencies, including the Department of Health, any private operator who is not complying with the screening or the required 50% capacity limitation, provided in this Executive Order.

Section 12: **GUIDELINES AND REGULATIONS.** The provisions contained herein may be defined, reinforced, and supplemented in detail through

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guidelines issued by any agency called upon to establish rules and regulations for the services discussed herein, including the Department of Health, the Human Resources Administration and Transformation Office of the Government of Puerto Rico, the Department of Labor and Human Resources, the Occupational Safety and Health Administration of Puerto Rico, the Department of Education and the Tourism Company, in conjunction with the Office of the Legal Advisor of the Governor. All agencies that promulgate guidelines in order to explain in detail the provisions of this executive Order shall publish them immediately and as broadly as possible.

Section 13: **TESTING CENTERS.** In order to facilitate the necessary COVID-19 monitoring and the compliance with this Executive Order, the Department of Health shall continue to facilitate testing to detect said virus, as established in Administrative Bulletin No. OE-2021-001. Said agency shall publish in electronic media, including the webpage of the Department of Health, the locations where testing is being conducted. Moreover, it is hereby order to continue disseminating educational material to raise awareness of the benefits of vaccination against COVID-19.

Section 14: **NONCOMPLIANCE.** Failure to comply with the provisions of this Executive Order by any person and/or business shall entail the imposition of the criminal penalties and fines established in Section 5.14 of Act No. 20-2017, as amended, which sets a penalty of imprisonment not to exceed six (6) months, or a fine of not more than five thousand dollars ($5,000), or both penalties, at the discretion of the court and/or any applicable law. Furthermore, in accordance with the provisions of Section 33 of the "Organic Act of the Department of Health," "[a]ny natural or juridical person who violates the provisions of this Act or the regulations issued by the Department of Health thereunder shall incur a misdemeanor, and upon conviction, may be sentenced to imprisonment that shall not exceed six (6) months, or a fine of not more than five thousand dollars ($5,000), or both

penalties in the discretion of the court." Moreover, subsection (b) of said Section provides that: "[a]ny natural or juridical person who violates the provisions of this Act or the regulations set forth by the Department of Health hereunder for the first time, shall be liable for an administrative fine of not more than five thousand dollars ($5,000), as provided in [Act No. 38-2017, the Government of Puerto Rico Uniform Administrative Procedure Act"]; in the case of a new violation of this Act or the regulations set forth by the Department by virtue thereof within the term of one (1) year, the fine imposed may be raised to a maximum of ten thousand dollars ($10,000)."

Any person who fails to comply with the provisions of this Order shall be subject to criminal prosecution, which may be initiated without delay by the Department of Justice, which, in turn, shall request the imposition of bail, as established in the Rules of Criminal Procedure.

Section 15: **SUPREMACY.** This Executive Order is not intended to be in conflict with any guidelines or orders issued by any federal agency. On the contrary, the provisions of this Act shall be interpreted in accordance with the federal provisions and the applicable case law on vaccination of employees of the public and private sector, as well as on the population in general.

Section 16: **REVIEW AND MODIFICATIONS.** The Government of Puerto Rico is constantly reviewing scientific data and the progress of each measure implemented. This Executive Order may be amended depending on the collected data and the results obtained, in order to adopt any modification as necessary to address a particular situation.

Section 17: **DEFINITION OF THE TERM AGENCY.** For the purposes of this Executive Order, the term "Agency" refers to any agency, instrumentality, office, or department of the Executive Branch of the Government of Puerto Rico, including public corporations, regardless of its name.

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Section 18: **NON-CREATION OF ENFORCEABLE RIGHTS.** This Executive Order is not intended to create any rights, substantive or procedural, enforceable at law or equity, by any person or entity, in any matter, civil, criminal, or administrative, against the Government of Puerto Rico or its agencies, officials, employees, or any other person.

Section 19: **SEVERABILITY.** The provisions of this Executive Order are separate and independent from each other, and if any part, section, provision, or sentence of this Executive Order is held to be unconstitutional, void, or invalid by a court of competent jurisdiction, such holding shall not affect the validity of the remaining provisions, which shall remain in full force.

Section 20: **REPEALING CLAUSE.** This Executive Order renders ineffective, upon its effectiveness, administrative bulletins nos. OE-2021-058, OE-2021-062, OE-2021-063, and OE-2021-064, and any other executive orders that are inconsistent, whether in whole or in part, with the provisions herein to the extent of such inconsistency. Furthermore, pursuant to Section 5.10 of Act No. 20-2017, administrative orders OA 508; OA 508A; OA 509; OA 509B; OA 512; OA 513; and OA 518B issued by the Department of Health and all those that are inconsistent with the provisions herein are hereby repealed. However, administrative bulletin nos. OE-2021-037 and OE-2021-073 shall remain in effect.

Section 21: **PUBLICATION.** This Executive Order must be filed immediately with the Department of State and the broadest possible publication is hereby ordered.

Section 22: **EFFECTIVENESS.** This Executive Order shall take effect immediately and shall remain in effect until it is rendered ineffective the emergency declared in Administrative Bulletin No. OE-2020-020, or until this Order is amended or repealed by a subsequent order or by law.

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CERIFIED TRANSLATION

IN WITNESS WHEREOF, I hereby issue this Executive Order under my signature and cause the Great Seal of the Government of Puerto Rico to be affixed, in La Fortaleza, San Juan, Puerto Rico, on this 15th day of November of 2021.

PEDRO R. PIERLUISI
GOVERNOR

Promulgated in accordance with the law on this 15th day of November of 2021.

OMAR J. MARRERO DÍAZ
SECRETARY OF STATE

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OFICINA DEL GOBERNADOR

Gobernador ordena vacunación a empleados de industria de salud y hospederías turísticas

(Con excepción de quienes presenten situaciones médicas o religiosas)

5 de agosto de 2021- El gobernador de Puerto Rico, Pedro R. Pierluisi, ordenó hoy en la **Orden Ejecutiva 2021-062** que todos los contratistas del gobierno que frecuenten de manera presencial su trabajo, así como todos los empleados que trabajen en sector de la Salud público o privado deben estar vacunados o contar con algunas de las exepciones. También, se ordena a todos los huéspedes de hoteles, paradores, hospederías o alquileres de corto plazo a presentar prueba de inoculación.

La Orden establece que todo contratista del gobierno que trabaje de forma presencial tendrá que presentar evidencia de vacunación o prueba negativa semanalmente. Los que se vacunen deben terminar el proceso en o antes del 30 de septiembre.

Como en la pasada Orden Ejecutiva contra el COVID-19, las excepciones serán las personas con condición médica cuya salud pueda perjudicarse, pero deberán tener un certificado médico a esos fines certificado por un profesional de la salud. Asimismo, personas que por motivos religiosos decidieron no vacunarse tendrá que ser certificado mediante declaración jurada del líder de su congregación o denominación religiosa. Sin embargo, deberán presentar pruebas negativas de COVID-19 o prueba positiva con certificado médico de recuperación.

"Como he dicho en otras ocasiones estamos ante una emergencia de salud pública mundial y está en cada uno de nosotros protegernos y por tanto proteger a nuestra comunidad. Todos tenemos que remar juntos en la misma dirección para vencer este virus. Estas estrategias de vacunación, así como las acciones del Departamento de Salud haciendo pruebas por todo Puerto Rico, llevando las vacunas a zonas remotas y exigiendo las mascarillas en espacios cerrados o en lugares donde existen aglomeraciones, son esenciales para combatir el COVID-19. Continuamos monitoreando el avance de los contagios y no descartamos tomar medidas adicionales, tales como limitar horarios de actividades públicas y comerciales o reducir la capacidad permitida en los establecimientos. Todos tenemos que cooperar", sentenció el gobernador.

Por su parte, el secretario de Salud, Carlos Mellado, "continuamos la lucha para hacerle frente al COVID-19. Mientras haya personas sin vacunar, vamos a continuar viendo escenarios como el de las últimas dos semanas; aumento de contagios y nivel de posibilidad alta. Sin embargo, en nuestra misión de salvaguardar la salud de todos los ciudadanos en la Isla nos dirigimos a tomar medidas importantes de seguridad. La exhortación es la inmunización mediante la vacuna contra el virus, lavado de manos, uso

[CERTIFIED TRANSLATION]

Governor orders vaccination of healthcare industry and hotel employees

8/5/2021

Health

(Except for those to whom medical or religious exemptions apply)

August 5, 2021- The Governor of Puerto Rico, Pedro R. Pierluisi, issued **Executive Order 2021-062** today mandating that all government contractors working in-person, as well as all employees in the public or private healthcare sector, must be vaccinated or be included in one of the exemption categories. All guests staying at hotels, *paradores*, inns, or short-term rentals are also required to present proof of vaccination.

The Order establishes that all government contractors working in-person must present proof of vaccination or weekly negative test results. Those who decide to get the vaccine must complete the process by September 30th.

As with the last COVID-19 Executive Order, an exception will be made for people with medical conditions whose health could be affected [by the vaccine], but they must obtain a medical certificate to such effect signed by a healthcare professional. Likewise, those who have decided not to get vaccinated for religious reasons must certify this by means of a sworn statement from the leader of their religious congregation or denomination. They must, however, present negative COVID-19 test results or a positive test result with a medical certificate of recovery.

"As I have said before, we are facing a global public health emergency, and it is up to each one of us to protect ourselves, hence protecting our community. We must all paddle together in the same direction to beat this virus. These vaccination strategies, as well as the actions taken by the Department of Health doing testing all around Puerto Rico, taking vaccines to remote areas, and requiring masks in enclosed spaces or crowded areas, are essential to fight COVID-19. We continue monitoring the spread of the virus and do not rule out taking additional measures, such as limiting the hours of public and business activities or reducing the occupancy allowed in the venues. We must all cooperate," the governor stated.

Similarly, Secretary of Health Carlos Mellado said that, "We are still battling COVID-19. As long as there are unvaccinated individuals, we will continue to see scenarios such as

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the one in the past two weeks: an increase in cases, and a high probability level. However, in our mission to protect the health of everyone on the island, we are set to take important safety measures. We are urging immunization through the vaccine against the virus, hand-washing, the use of masks, distancing, and protecting ourselves in order to take care of our people. It is our responsibility to the community. We want to control the pandemic, but we need everyone's cooperation."

The secretary of health announced an amendment to Regulations No. 138-A to require the presentation of the COVID-19 vaccination card in order to obtain a health certificate in Puerto Rico. "As of today, anyone who needs a health certificate must be vaccinated against COVID-19 to obtain it."

In the case of health facilities, vaccination—or, alternatively, negative COVID-19 test results—will be required of all employees. This includes, but is not limited to, hospitals, clinical laboratories, medical offices, healthcare and therapy centers, blood banks, [and] pharmacies. This requirement also applies to anyone who works at senior daycare or long-term care centers.

Regarding the tourism industry, guests at hotels, *paradores*, or inns, including short-term rentals through platforms such as AIRBNB, VRBO, [and] Join-a-Join, among others, will likewise have to present proof of vaccination or negative COVID-19 test results. Tourists who arrive in Puerto Rico with negative COVID-19 test results whose stay is longer than one week will have to get another test.

This requirement applies to employees of hotels, *paradores*, and inns, who must have completed their vaccination cycle by September 30th. In fact, Pierluisi and Mellado recommended that those licensed to run these establishments apply rules similar to this one to their employees.

Each agency or company will be responsible for ensuring compliance with this Order, and failure to comply will entail fines [sic] of up to six months in jail, fines of up to $5,000, or both, at the discretion of the Court.

"We reiterate our recommendation that other branches of the government, public corporations, municipalities, and private and business establishments voluntarily adopt this Executive Order," Pierluisi concluded.

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DEPARTMENT OF
Health

Government of Puerto Rico
Health Department

Regulation of the Secretary of Health No. 138-A to amend the Regulation of the Secretary of Health No. 138 for the Issuance of Health Certificates in Puerto Rico

Number: 9295
Date: August 5th of 2021
Approved: Omar J. Marrero Díaz
Secretario de Estado

Government of Puerto Rico
Health Department

Regulation of the Secretary of Health No. 138-A

Amendment to the Regulation of the Secretary of Health No. 138, Regulation for the issuance of health certificates in Puerto Rico, Regulation No. 7784 of December 9, 2009, as registered in the Department of State.

Article 1: Legal Basis

Regulation of the Secretary of Health No. 138, Regulation for the issuance of health certificates in Puerto Rico, Regulation No. 7784 of December 9, 2009, as registered in the Department of State (Regulation No. 138), and is promulgated by virtue of Act No. 81 of March 14, 1912, as amended, better known as the "Organic Law of the Department of Health," Act No. 38 of June 30, 2017, as amended, better known as "Uniform Administrative Procedure Act of the Government of Puerto Rico" and Act No. 232 of August 30, 2000, known as the "Puerto Rico Health Certification Act."

Article 2: Purpose

These amendments are adopted with the purpose of expressly establishing the requirement to present the vaccination card against COVID-19 or the "COVID-19 Vaccination Record Card" as an essential document for a doctor to issue a health certificate.

As indicated below, Article IV is amended to add subsections (s) and (t) of Regulation No. 138. Also, subsection (1) of Article X and subsection 4 (A) is added to Article X of Regulation No. 138.

Article IV is amended. Definitions, to add the following subsection:

s. CDC: Disease Control and Prevention of the United States Department of Health.

t. "COVID-19 Vaccination Record Card": official vaccination card against COVID-19 issued by the CDC, which identifies individuals who have been completely inoculated with the aforementioned virus. It is the proof or supporting evidence that an individual is vaccinated or inoculated.

Article X is amended. Tests required to issue a health certificate to read:

1. No doctor may issue health certificates without the following: (1) a medical evaluation, (2) having certified that the person has shown evidence of vaccination against COVID-19 (COVID-19 Vaccination Record Card) with the series of complete vaccine, issued by the CDC, (3) the results of the in vitro tuberculin or tuberculosis test and (4) the serological test for syphilis, with their respective confirmatory tests when applicable.

As an exception, a doctor may issue the health certificate without the person being inoculated with the COVID-19 vaccine in those cases where the patient has a compromised immune system or there is a medical contraindication that prevents inoculation. This must be certified by a doctor authorized to practice in Puerto Rico or by the doctor who issues the Health Certificate. In addition, the doctor must certify the duration of the medical contraindication and whether it is temporary or permanent. If it were temporary, once the contraindication ceases, the person must comply with the vaccination requirement, for subsequent Certificates.

On the other hand, it is allowed - by way of exception - that the Health Certificate be issued to people not inoculated for religious reasons, as long as the vaccine goes against the dogmas of the patient's religion. The doctor must certify that he was shown the sworn statement required by the Department of Health for these cases, in accordance with the Executive Orders in force.

4 (A) The doctor will require the original vaccination card, as well as a legible copy of it in order to prove its validity. For high-risk patients, the licensed physician may require a negative COVID-19 result from a qualified SARSCoV2 viral test (nucleic acid amplification tests (NAAT) or antigen tests).

Article 3: Validity

This Regulation shall take effect immediately, by virtue of Section 2.13 of Act No. 38-2017, as amended, known as the "Uniform Administrative Procedure Act of the Government of Puerto Rico" (3 LPRA S 9623).

In San Juan, Puerto Rico, today August 5, 2021.

Dr. Carlos R. Mellado López
Secretario de Salud