COVID-19 in Irish Hospital Healthcare Workers; SARS-CoV-2 antibody prevalence, epidemiology of infection and antibody response to vaccination

Medical Doctorate, 2023

Trinity College Dublin, the University of Dublin

Dr. Niamh Allen

Supervisor: Professor Colm Bergin

Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work. This thesis is based on a national study, for which I was the principal investigator. A study steering committee was formed to guide the study; the thesis includes both published and unpublished work that was undertaken by this study group, all of which was led by me. I am the lead author on all published work that has resulted from this research, some of which is included in this thesis. The individual contributions of others are detailed in the acknowledgement section and in the section entitled "My role in this research". Informed consent was obtained from all participants involved in this research.

I agree to deposit this thesis in the University's open access institutional repository or allow the library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement. I consent to the examiner retaining a copy of the thesis beyond the examining period, should they so wish (EU GDPR May 2018).

Niamh Allen

Date

Acknowledgements

I would like to acknowledge Professor Colm Bergin and Professor Catherine Fleming who helped to design and implement this research as lead site investigators in St. James's Hospital and University Hospital Galway respectively, and who have both supported me personally and professionally throughout this research period, and for many years beforehand. I would like to thank the National Public Health Emergency Team (NPHET) for endorsing this research. I would also like to thank the research steering group who helped plan each sub-study and critically evaluated reports that were generated as part of this study for feedback to NPHET and for wider dissemination in the form of scientific manuscripts; this steering committee was led by Dr. Lorraine Doherty. Data for this study was collected and processed by numerous individuals; Dr Una Ni Riain (consultant microbiologist in University Hospital Galway) and Professor Niall Conlon, consultant immunologist in St. James's Hospital helped to design, facilitate and coordinate the laboratory testing conducted as part of this research. I would like to thank my co-members of the data-analysis sub-group; Professor Cathal Walsh (University of Limerick) provided statistical support to the research; Lisa Domegan, Melissa Brady and Annamaria Ferenczi of the Health Protection Surveillance Centre (HPSC) and Antonio Isidro Carrion Martin (University of Murcia and epidemiologist with MSF Ireland) provided epidemiological support for this research. I would also like to acknowledge the research team at each hospital site (Shane Walsh and Tracey Kinsella in SJH and Claire Heavin and Peadar Rooney in UHG) who helped to coordinate the running of each sub-study in each hospital. The hospital management of both hospitals were extremely supportive of the study from the outset, and without their support the research could not have taken place. I thank them for this, as well as all staff of both hospitals who participated. I would especially like to acknowledge the phlebotomy departments in each hospital for facilitating the sampling of almost 6000 participants on two separate occasions, the microbiology, virology and biochemistry laboratories in each hospital for processing these samples on two different assays, including doing all of the assay validation necessary for the implementation of new assays not previously used in the hospitals, and the National Virus Reference Laboratory (NVRL) for providing additional testing on certain blood samples. The human resources departments assisted with denominator data and the occupational health departments provided data on COVID-19 positivity rates among staff at different points during the pandemic.

Isidro and Alba put up with me undertaking this research at a very busy time in our lives and dealt with unplanned absences. Luan put up with me working until a few hours before he was born. He was less tolerant out of utero but Nana came to the rescue, as she has been doing my whole life.

Table of Contents

Chapter 3: Seroprevalence of antibodies to SARS-CoV-2 in Hospital Healthcare Workers, October 2020; a cross-sectional study (PRECISE 1)

Chapter 4: Comparison of three commercially available assays for the detection of SARS-CoV-2 antibodies in Hospital Healthcare Workers

Chapter 7: Summary, Conclusion and Recommendations

My role in this research

Study inception and design

As the principal investigator for this research, I was involved in the concept and evolution of the studies. This was a national research consortium, instigated and designed by me and a scientific research group – the study steering committee described below. The research programme was endorsed by the National Public Health Emergency Team (NPHET) and a study steering committee was set up to oversee the proper conduction of each sub-study. This committee consisted of members of the Health Service Executive (HSE), public health specialists, microbiology, immunology and virology experts, occupational health physicians and infectious disease physicians from each study site. I led the overall aims of the research collaborative and the individual design of each sub-study, with the support of this study steering committee, for all sub-studies detailed in this thesis. This included leading discussions on re-designing the research and incorporating new sub-studies to reflect new and emerging evidence in the dynamic setting of a pandemic. Along with the site coordinators, I sought local support for the research from the hospital management at each study site. I designed the research questionnaires and wrote the study protocols. I researched and organised the online platform, which was used to consent participants, and where the questionnaire was completed by participants. This included liaising with the provider to communicate our needs, and providing the link between the hospitals, the HSE and the online provider to navigate GDPR-related queries. I learned to use this online platform, including its unique coding system, to compose the questionnaires for individual sub-studies, and to facilitate smooth transition through the questionnaire with dependent variables. I organised testing of the questionnaire in order to ensure the smooth running of each study. I made a data analysis plan at the outset to ensure that the questionnaire variables would easily lend themselves to appropriate statistical analysis. Along with the online provider, I helped to design the research website and flow. I created all text for the website and coordinated agreement among the research team. I worked closely with the HSE communications department to aid the design of an appropriate logo for the study and designed the research materials (consent form, participant information leaflets, posters and pull-up stands) and organised their translation into the five languages most widely spoken in both hospitals. I worked with a separate information technology (IT) company (Swiftqueue) to set up a system for participants to book blood tests. This involved setting up a system whereby a unique identifier code (MRN) would be generated by the booking; this MRN would be produced in the form of a barcode sticker for the blood sample in order to ensure anonymous processing of blood tests in the laboratory. I coordinated with the laboratory in each site, Swiftqueue management, and the head phlebotomists in order to ensure this barcode design was functioning from printing to barcode reader in the laboratory. This also involved learning about the different types of barcodes that would be readable on different analytic platforms.

Funding

Along with the research coordinator and key members of the steering committee I put together a proposal for funding for this research programme. This involved cost assessments for both sites which had to be estimated by phlebotomy, laboratory and human resources departments for consumable and non-consumable items, and salaries for a study team of two at each site. Funding was gained through the HSE COVID-19 budget, to the effect of e665.116. I have been the main person responsible for tracking the expenditure of this research programme, along with the finance departments at each site. I have given financial feedback at steering group meetings and have led discussion about movement of finances as the research has evolved. In the research funding we approved a full-time role of senior project manager, however in the short time available to get the research programme up and running we were unable to fill this post, and I took on the majority of the organisational work of this position.

Ethical Approval

I lead the national ethical approval process for the research, including amendments as the evolving nature of the pandemic led to alterations in sub-studies. This was done through the National Office for Research Ethics Committee for COVID-19 (NREC). I completed all data protection impact assessments (DPIAs) and data-sharing agreements necessary for the proper ethical approval and running of the individual studies of the research programme and liaised with the data protection officer (DPO) at each site and within the HSE to ensure all data acquisition and processing met GDPR regulations and ethical requirements. With the roll-out of vaccination I applied for amendments to our ethical approval to ensure the research programme could adapt to inform in the changing setting of a pandemic.

Human Resources

Along with the CEO and lead site investigator, I prepared job descriptions and interviewed prospective candidates for the research team roles in St. James's Hospital, where I was based. The lead site investigator in University Hospital Galway led this process in Galway.

Committee membership, collaboration, management and leadership

As principal investigator for this national research programme, I took a leading role in the study steering committee. I ensured feedback at all stages of the study to this committee. I also lead the research teams at each site and collaborated with senior management (CEO/DCEO) at each site in order to run each study. Due to the urgency of the initiation of the research in the first six months of the pandemic, the research teams were only in place a matter of weeks before the commencement of the phlebotomy component of the first sub-study, which meant that a lot of support was needed in this set-up phase. This was provided by me and by the lead site investigator at both sites. I was predominantly based in SJH for the duration of the study, but this also involved trips to Galway to support the study team there. I liaised personally with the laboratories at each study site to

ensure smooth processing of samples from phlebotomy through laboratory. I dealt with all issues that arose logistically and technologically during the research and had a very "hands-on" approach to the running of each study. This included stepping in to phlebotomise where gaps arose unexpectedly in the phlebotomy rota. I dealt personally with any complaints arising from participants. I dealt with any errors on the part of the research team, including one accidental data breach. I set up a data-subgroup to help coordinate and support the analysis of the data. This involved finding statistical and epidemiological support from outside of the steering committee. I coordinated and led the results management at both sites, setting up virtual clinics for positive results management and for medical call-backs to any participant requesting to speak with a medical member of the team. I was also involved in personally making these phone calls to discuss individual results with participants.

I worked with other study teams working in the area of COVID-19 to ensure as much collaboration as possible in order to maximise outputs. This included members of the DIRECTS (Detecting Innate Protection against SARS-CoV-2) study team, who requested input on the online component of PRECISE 1 and 2 sub-studies, advice on recruitment, sharing of recruitment communications etc. I have also worked with a team from the HPSC to develop a vaccine efficacy study (as part of a WHO protocol used across Europe to maximise strength of results). I have contributed experience from the PRECISE research programme and have aided cost-assessment based on the costings of the PRECISE studies to date.

Communications

I lead the study teams and steering group on all communications pertaining to this research. This included both national and local communications. I worked closely on the HSE communications team on all national communications, including announcement of the research and dissemination of results. I worked closely with local communications at each site to ensure the same at local level. This was especially pertinent in the run up to the first study, in order to maximise recruitment at a time when mass gatherings of staff were not possible due to COVID-19 restrictions. I communicated closely with senior hospital management to ensure they were the first to be aware of sub-study results prior to national dissemination.

I have led the research team at each site to issue personal results to all participants. I dealt personally with any difficult communications with participants, for example those who were upset by an unexpected result, and those who incorrectly filled out study questionnaires with data that could not subsequently be confidently matched to laboratory results.

I have coordinated between the steering committee, the data sub-group, the microbiology, virology and biochemistry laboratories at each site, and members of staff of the national virus reference laboratory (NVRL) to ensure agreement on the wider dissemination of aggregate results, including the scientific manuscripts contained in this thesis, and reports to NPHET.

Laboratory Analysis

I coordinated with key stakeholders in the laboratories at each site to organise the on-site testing of the samples. I liaised with designated laboratory management and finance departments to organise the purchasing of the assays on the study account to ensure appropriate reimbursement. I provided a liaison between Swiftqueue and the laboratory to ensure flow of samples and barcodes from phlebotomy sites to the laboratory. I was not involved in any of the actual testing of the samples, or validation of the laboratory assays. This was done in its entirety by the laboratory staff.

Database management and statistical analysis

I designed the database for data collection and storage for each sub-study in this research programme. I organised the safe storage of the data and performed the pseudo-anonymisation of the data when appropriate. I matched the blood test results via MRN to the named data of the participants on the blood booking system. I then further matched them to their questionnaire data using statistical software. Following the second round of blood testing I also matched participants common to both studies in order to conduct the longitudinal analysis. I conducted basic analysis on the data, including study participant characteristics, basic seroprevalence by site, role and demographics. Advanced analysis such as the univariate and multivariable analysis was conducted by members of the data sub-group, which was coordinated by me. Namely these were Annamaria Ferenczi for the data presented in chapter 3 and Melissa Brady for the data presented in chapters 4-7. Isidro Carrion Martin and Lisa Domegan supported Annamaria and Melissa for this data analysis. I sourced advanced statistical knowledge when required; this was provided by Cathal Walsh, who provided statistical advice regarding all data presented in Chapters 3-7. Where not performing the analysis myself, I lead all discussions as to what was required of the analysis, and during analysis the data-subgroup met at least once weekly to discuss the direction of the analysis. These discussions were organised and led by me.

The set of research studies described in this thesis were conducted at a moment in time in the COVID-19 pandemic in Ireland. The subsequent emergence of new Variants of Concern (VOCs), most notably the Omicron variant, have changed the landscape of the pandemic, and The PRECISE research continuum remains in place with further sub-studies ongoing (with a new principal investigator) to address the real time research questions that continue to be raised in this changing landscape, as described in Chapter 7, Next Steps.

List of Tables and Figures

Chapter 5

Chapter 6

Appendix 1 – Tables and Figures

Chapter 3

Appendix 2 - Publications based on this research

List of Abbreviations

HCW – hospital healthcare workers

COVID-19 – coronavirus disease of 2019

SARS-CoV-2 - severe acute respiratory syndrome coronavirus 2

PRECISE Study (Prevalence of Covid-19 in IriSh hEalthcare workers)

SJH – St. James's Hospital, Dublin

UHG – University Hospital Galway

ELISA - enzyme-linked immunosorbent assay

RR – relative risk, aRR – adjusted relative risk

95% CI – 95% confidence interval

PCR – polymerase chain reaction

SCOPI – Study to Investigate COVID-19 infection in People Living in Ireland

Ig – Immunoglobulin

Abbott – Abbott SARS-CoV-2 anti-nucleocapsid IgG antibody assay

Roche – Roche Elecysis SARS-CoV-2 anti-nucleocapsid panantibody assay

Wantai – Wantai ELISA SARS-CoV-2 anti-spike protein antibody assay, distributed by Fortress Diagnostics

EMA – European Medicines Agency

VOC – Variant of Concern

Pfizer Vaccine - BNT162b2mRNA – BioNTech

Moderna Vaccine - mRNA-1273

Vaxzevria Vaccine - ChAdOx1 nCOV-19 (Vaxzevria - formerly COVID-19 Vaccine AstraZeneca)

J&J/Janssen Vaccine - Johnson & Johnson's Janssen COVID-19 Vaccine

NIAC – National Immunisation Advisory Committee

B-cell – bursa-derived lymphocyte

T-cell – T (thymus) lymphocyte

mRNA – messenger ribonucleic acid

HCA – healthcare assistant

NVRL – National Virus Reference Laboratory

NREC – National Office for Research Ethics Committee

HPSC – Health Protection Surveillance Centre

HSE – Health Service Executive

NPHET – National Public Health Emergency Team

Anti-N antibody – anti-nucleocapsid antibody

Anti-S antibody – anti-spike antibody

PPE – personal protective equipment

IT – information technology

Context for the research

This research was conducted during the first three waves of the COVID-19 pandemic in Ireland. The research programme was designed during the first wave of the pandemic in Spring 2020, and the final sub-study took place in the latter stages of the third wave in April 2021. Due to the rapidly evolving nature of the pandemic, the understanding of COVID-19 infection, the mechanisms of transmission, the effectiveness of vaccination, the impact on immunity and the role of emerging therapeutics advanced significantly in the almost two years since completion of the research programme described in this thesis. The PRECISE research programme is ongoing, as described in chapter 7, and continues to inform national and international understanding, however it is important to contextualise the part of the research described in this thesis in order for the reader to understand its contribution to national, and indeed international, understanding of the situation at the time. There are four main changes to the COVID-19 landscape that occurred after the completion of the research described in this thesis; the first of these was the emergence of the Omicron variant of concern (VOC), which was the most challenging of any of the VOCs to date and spurred the largest global surge of COVID-19 in early 2022, representing the biggest change in the nature of transmission of the virus. The second was the advent and impact of vaccination; the first COVID-19 vaccines were approved during the first part of our research, and while we modified the second sub-study to take this into account, we did not yet have any real world understanding of the effect of or durability of response to vaccination on infection presentation or on reduction of transmission, nor was the duration of protection known – the need for booster vaccine programmes had not yet been determined. The third factor that changed the landscape of the pandemic was the development of treatment strategies which also post-date the completion of the research described in this thesis. A fourth change, which evolved as a result of a combination of factors including the advent of treatment and vaccination, has been the move away from strict isolation of contacts and testing of all cases, and the move away from government-enforced mass restriction of population movement. It is important to understand that this research was conducted in a landscape of sequential strict lockdowns on public movement, 14-day isolation of cases, quarantine of all close contacts, and testing of all close contacts in an effort to identify each and every case of COVID-19 infection.

Abstract

Background

Hospital healthcare workers (HCW) are at increased risk of contracting COVID-19 infection. We aimed to determine the seroprevalence of SARS-CoV-2 antibodies in HCW in Ireland in two locations with diverging community and HCW incidence at two points in time during the COVID-19 pandemic, and to determine HCW risk factors for seropositivity. We also aimed to compare commercially available assays for the detection of SARS-CoV-2 antibodies, and to measure serological response to COVID-19 vaccination. Two tertiary referral hospitals in Irish cities with diverging community incidence and seroprevalence were identified; COVID-19 had been diagnosed in 10.2% of staff of St. James's Hospital, Dublin (SJH) and 1.8% of staff of University Hospital Galway (UHG) respectively by the time of the first study (October 2020 – PRECISE 1). PRECISE 1 took place prior to the roll-out of COVID-19 vaccination. The second study (PRECISE 2) took place in April 2021, four months after the start of vaccination at both sites.

Methods

Two cross-sectional studies were performed in October 2020 and April 2021 respectively. All staff of both hospitals (N=9038) were invited to participate in an online questionnaire and blood sampling for SARS-CoV-2 antibody testing in October 2020 and April 2021. In October 2020, all samples were tested on two serological assays (Abbott Architect IgG assay and Roche Elecsys panantibody assay, both detected antibodies to the anti-nucleocapsid (anti-N) protein) and a subset of samples were also tested on the Wantai ELISA total antibody assay, targeting antibodies to the anti-spike (anti-S) protein. In April 2021, all samples were tested via the Roche Elecsys assay for both anti-N and anti-S antibodies. Frequencies and percentages for positive SARS-CoV-2 antibody were calculated and adjusted relative risks (aRR) for participant characteristics were calculated using multivariable regression analysis. The performance of each assay was compared.

Results

In October 2020 5,788 HCW participated (64% response rate). Seroprevalence of antibodies to SARS-CoV-2 was 15% and 4.1% in SJH and UHG, respectively. Thirty-nine percent of infections were previously undiagnosed. On multivariable analysis, the adjusted relative risk (aRR) for seropositivity was higher for healthcare assistants (aRR: 2.0, 95%CI:1.4–3.0), nurses (aRR: 1.6, 95%CI: 1.1–2.2), daily exposure to patients with COVID-19 (aRR: 1.6, 95%CI: 1.2-2.1), age 18-29 years (aRR: 1.4, 95%CI: 1.1-1.9), living with other HCW (aRR: 1.3, 95%CI: 1.1–1.5), Asian background (aRR: 1.3, 95%CI: 1.0-1.6), and male sex (aRR: 1.2, 95%CI 1.0-1.4). In HCW with prior PCR-confirmed COVID-19 infection (N=367), the Roche assay detected 95% of these infections, and had sustained positivity up to 33 weeks after infection. Only 41% of these participants tested positive on the Abbott assay. The decline in Abbott positivity starting at week 21/day 150 after confirmed infection.

In April 2021 5085 HCW participated (56% response rate). Seroprevalence of antibodies to SARS-CoV-2 indicative of past infection rose to 21% and 13% in SJH and UHG respectively. Twenty-six percent of infections had been previously undiagnosed (decreased from 39% in October 2020). Risk of seropositivity was higher for the following characteristics: working in SJH (aRR 1.5, 95% CI 1.3-1.8, p<.001), being a healthcare assistant (aRR 1.8, 95% CI 1.3-2.3, p<.001), being of African or other black background (aRR 1.7, 95% CI 1.3-2.2, p<.001), lower level of education (aRR 1.4 for secondary level education, 95% CI 1.1-1.8, p=0.002), being a nurse (aRR 1.4, 95% CI 1.0-1.8, p=0.022), daily contact with COVID-19 patients (aRR 1.4, 95% CI 1.1-1.7, p=0.002), daily contact with patients without suspected or confirmed COVID-19 (aRR 1.3, 95% CI 1.1-1.5, p=0.013), being 18-29 years of age (aRR 1.3, 95% CI 1.1-1.6, p=0.002), being male (aRR 1.2, 95% CI 1.0-1.4, p=0.016), and living with other HCW (aRR 1.2, 95% CI 1.0-1.4, p=0.007). One hundred percent of fully vaccinated participants had detectable anti-spike antibodies in response to vaccination. Twenty-three of these had had breakthrough COVID-19 infection, representing 0.6% of all fully vaccinated participants.

Conclusion

The HCW seroprevalence was six times higher than community seroprevalence following the first wave of the pandemic in Ireland and rose further with subsequent waves. Risk was higher in the hospital situated in a higher density area with higher community incidence during the three waves of the pandemic that occurred during this research. Risk was higher for those with close patient contact. The proportion of undiagnosed infections decreased from October 2020 to April 2021 but remained high; this calls for ongoing robust infection control guidance and easy access to testing for HCW. Screening of asymptomatic HCW can help to decrease transmission, inform vaccine efficacy surveillance, and enhance infection prevention and control (IPC) measures, however the role of such screening remains to be clearly defined. The Roche assay performed better than the Abbott assay at detecting past infection and is more appropriate for future population-based studies looking at seroprevalence post natural infection. With emerging evidence of reduction in transmission from vaccinated individuals, the authors strongly endorse rapid vaccination of all HCW. The production of antibodies post-vaccination in all participants fully vaccinated is extremely reassuring; further studies are needed to correlate this serological response with functional immunity. The proportion of breakthrough infections in fully vaccinated participants was in keeping with the published literature; formal vaccine efficiency studies are needed to further characterise breakthrough infections and estimate protection from infection, particularly with the ongoing emergence of variants of concern. The results of this research and the lessons learned from planning and executing the research can inform future pandemic planning as it pertains to hospital practices and risk factors for infection in HCW. This research platform has evolved with the evolution of the pandemic, with further sub-studies providing ongoing contribution to advancing our understanding of the changing epidemiology of COVID-19, and of infection and re-infection as relates to vaccination and serological status among the HCW cohort.

Lay Abstract

COVID-19 infection is a viral illness caused by a new coronavirus SARS-CoV-2, that has spread to become a global pandemic. Healthcare workers are more at risk of becoming infected by SARS-CoV-2 than most other workers; they can pick up the infection from patients or other healthcare workers who don't know they have the infection. They can pass this infection on to other staff or to patients and they remain working even in extreme situations such as lock down. When a person becomes infected with a new virus, their immune system becomes activated. In many cases specific antibodies against the new virus are produced. These antibodies can be detected in the blood, usually a week or two after the illness started. By doing a blood test for these antibodies it is possible to say that the person has been infected at some stage with the SARS-CoV-2 virus.

The aim of this study, called the PRECISE Study (Prevalence of COVID-19 in Irish Healthcare Workers), was to see how many healthcare workers in Irish hospitals have likely been infected with the SARS-CoV-2 virus, and to see changes in the prevalence of antibodies at two points in time during the pandemic. We aimed to compare the amount of infections (called the seropositivity) in two different areas of Ireland, one where there were more COVID-19 infections (Dublin) and one where there were less (Galway). There are many different tests for antibodies – called assays. We used two different types of assay for this test so we could also try to understand which one was better at picking up past infections. In April 2021 we also tested for a type of antibody that is produced after vaccination, so we were able to tell if staff had had an antibody response after their COVID-19 vaccination. This hopefully is a good indicator that the vaccine has made the person immune to severe COVID-19 disease, though we do not yet know yet how long that immunity will last for, and whether repeated vaccination will be necessary. The study was carried out in St. James's Hospital (SJH) in Dublin, and University Hospital Galway (UHG). All staff of both hospitals (>9000 people) were invited to participate by doing a short online questionnaire and giving a blood sample. This was done in October 2020 and again in April 2021.

Over 5000 staff members participated in October 2020 and in April 2021. The overall seroprevalence (the proportion of staff that had SARS-CoV-2 antibodies) was 15% in SJH and 4.1% in UHG in October and rose to 21% in SJH and 13% in UHG by April 2021. The difference between the two hospitals was expected, as in the community there was much higher level of transmission in Dublin than in Galway, and this is one of the factors that puts healthcare workers at risk. The difference between October 2020 and April 2021 was also expected, and it reflects the magnitude of the 3rd wave of the pandemic in both places. Other than location, there were several factors that meant HCW were more likely to have a positive antibody test such as male sex, Black or Asian ethnicity, and having jobs that involved close contact with patients, such as healthcare assistants, nurses and doctors. We also found that living with other HCW added to this risk, which confirms what we know about many of the infections happening in the household and not just in the hospital workplace. In Dublin, HCW are more likely to be living together in smaller spaces, and this may have contributed to the higher seroprevalence in the Dublin hospital. We found that in October almost 40% of people who had antibodies to COVID-19 had never

been diagnosed with COVID-19 infection. By April this had come down to 26%, but this is still high. Most staff had been fully vaccinated by April; all staff who had been fully vaccinated had antibodies produced as a reaction to the vaccine.

The study has helped in making decisions about how to control the spread of the infection in hospitals, who is most at risk and how to make the hospital a safer working environment. It has also helped to understand which tests are best to use when doing studies of this nature in the future, and to understand antibody response after COVID-19 vaccination. The fact that many infections had not been diagnosed means that it is important to keep following all of the guidelines for infection control and prevention, and we need to have easy access to testing for HCW. The antibody response to vaccination was excellent. Vaccine efficiency studies are ongoing worldwide, including in Ireland, and will help us understand better the link between vaccination, immunity and transmission. This research and other studies that have continued on from it will also help us to be better prepared for future pandemics.

Research Aims

The purpose of the research was to determine the prevalence of anti- SARS-CoV-2 antibodies in HCW in two hospitals with diverging community and healthcare rates of infection to improve understanding of HCW risk factors (demographic, living arrangements and work-related risks) for SARS-CoV-2 infection and to inform risk reduction activities and help health services to prepare for further waves of the pandemic. This research was planned in Spring/Summer 2020 following the first wave of SARS-CoV-2 and the research questions I wanted to answer at that time were:

- What is the seroprevalence of SARS-CoV-2 antibodies in HCW in Ireland following the first wave of infection?
- What are the main risk factors for SARS-CoV-2 acquisition in HCW in Ireland?
- How does this differ in locations of higher versus lower community incidence of COVID-19 infection?
- What proportion of COVID-19 infections in healthcare workers have been asymptomatic and/or undiagnosed?
- Have the seroprevalence, risk factors, and proportion of undiagnosed infections changed over the course of the pandemic as access to testing and recognition of symptoms has changed?
- What is the best commercially available SARS-CoV-2 antibody assay for future population seroprevalence studies such as this?
- How have antibody levels declined over time?
- What serological response have HCW had to COVID-19 vaccination?
- What proportion of HCW have had a proven COVID-19 infection post-dating receipt of vaccination?

Specific Objectives:

- To measure the prevalence of antibodies to SARS-CoV-2 in all healthcare workers in two hospitals in Ireland in areas of differing COVID-19 incidence relating to the first, second and third waves of the pandemic in Ireland.

- To compare the prevalence of antibodies to SARS-CoV-2 across the two geographical areas with higher and lower incidence of cases of COVID-19 in Ireland, as defined at the time of commencing the research.
- To perform the cross-sectional sero-survey at two time points during the pandemic and document the changing patterns in the prevalence of antibody positivity.
- To compare antibody prevalence by HCW demographics, working role, degree of patient exposure, and living arrangements.
- To examine the relationship between the presence of antibodies to SARS-CoV-2 and the self-reporting of symptoms consistent with COVID-19 or having a previous diagnosis of COVID-19 infection.
- To compare commercially available laboratory assays for the detection of antibodies to SARS-CoV-2
- To evaluate decay in SARS CoV2 antibody levels over a six-month time period.
- To evaluate antibody response to vaccination in vaccinated individuals.

Value of the Research

This research answered important questions about transmission and immunity dynamics of COVID-19 infection in the hospital setting in Ireland. These learning points can inform future pandemic planning as pertains to hospital and HCW practices and risk factors for infection. Prior to this study, we had an estimate of national community seroprevalence to SARS-CoV-2 from the SCOPI study, discussed in Chapter 1. The seroprevalence among HCW was presumed to be higher, based on HPSC incidence data, however it had not been calculated, and we did not know what the gap was between diagnosed and undiagnosed infections in HCW. Seroprevalence studies are being conducted worldwide with varying results in HCW, and therefore having our own Irish data on this was paramount. We also know that much onwards transmission is happening in Irish hospitals, contributing to local outbreaks both in hospitals and in the community. Understanding these transmission dynamics are key to controlling the hospital acquisition and outbreaks at national level, and this study has helped to identify the main risk factors related to HCW demographics, working role and living situation. The study has also confirmed the suspicion that seroprevalence among HCW are much higher than community seroprevalence, and at a national level this data has helped with the roll-out of the vaccination programme, confirming that HCW should be offered vaccination as early as possible, that all efforts should be made to achieve high vaccination rates among HCW, and that messaging among certain groups of HCW should be prioritised. At the outset of this research, there were very few published comparative data on the use of commercially available assays for detection of SARS-CoV-2 antibodies and on the duration of antibody response after confirmed infection. Our research studies have contributed to national and international understanding on both of these fronts. Finally, when this study was designed we did not yet have vaccination against COVID-19 infection. We were able to adapt the second study (PRECISE 2) to measure antibody response to vaccination, which was an emerging field at the time of our research; the research programme was designed prior to the advent of SARS-CoV-2 vaccination. This helped at a local level to quantify the proportion of staff expected to be immune, not just by history of vaccination, but also by proven antibody response. This has also added to the understanding of the relationship between antibody response (either following natural infection or vaccination) and immunity, which is yet to be clearly established, especially regarding VOC. Of note, this set of studies was conducted prior to the emergence of the omicron VOC, but it has fuelled further research, including partnership in a WHO vaccine efficacy (VE) study which is ongoing. This research has also led to further studies as described in Chapter 7.2, highlighting the ongoing contribution of this research consortium after the completion of the work described in this thesis.

Outputs

The work detailed in this thesis has been published in report format and in scientific manuscripts and has been presented at meetings and conferences, as detailed below. I have won an annual award from the Royal Academy of Medicine in Ireland for the best oral presentation of work of epidemiological and /or public health interest by a person in a training post and by those undertaking research up to the level of PhD. Over the years this medal has been won by many of those currently prominent in public health in Ireland and is a well-recognised marker of distinction in epidemiology. I have also won a prize for the best oral presentation of research at the Infectious Diseases Society of Ireland National Conference.

Reports

1. Summary Report to National Public Health Emergency Team (NPHET) – 30th December 2020, with particular focus on at-risk groups for vaccination prioritisation.
2. Full Report of PRECISE 1 findings of October 2020 to NPHET 19th January 2021, available on HPSC website at https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/research/precise/PRECISE%20Study%20Phase%201%20Interim%20Report%20January%202021.pdf
3. Full Report of PRECISE 2 findings of April 2021 to NPHET July 2021, available on HPSC website at https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/research/precise/PRECISE%202%20Report%20v2.0%20with%20addendum.pdf

Publications in Scientific Journals (see Appendix 2 for full articles)

1. **Allen, N**., Ni Riain, U., Conlon, N. et al. (2021). Prevalence of Antibodies to SARS-CoV-2 in Irish Hospital Healthcare Workers. *Epidemiology and Infection*, 1-33. doi:10.1017/S0950268821000984.
2. Kerr C, **Allen N**, Hughes G, Kelly M, O'Rourke F, Lynagh Y, Dunne J, Crowley B, Conlon N, Bergin C (2021). SARS-CoV-2 anti-nucleocapsid assay performance in healthcare workers at baseline and 6 months. Irish Journal Medical Science. 2021 Jul 7:1–4. doi: 10.1007/s11845-021-02700-5. Epub ahead of print. PMID: 34235708; PMCID: PMC8262428.
3. **Allen N**, Fleming C, Bergin C (2021) Epi Insight, Volume 22, Issue 2, March 2021, Antibodies to SARS-CoV-2 in Hospital Healthcare Workers in Ireland. https://ndsc.newsweaver.ie/4otaa688p3/16yk7vqbqqt?lang=en&a=1&p=58872704&t=31302934

4. **Allen, N**., Brady, M., Carrion Martin, A. I., Domegan, L.et al. (2021). Serological markers of SARS-CoV-2 infection; anti-nucleocapsid antibody positivity may not be the ideal marker of natural infection in vaccinated individuals, Journal of Infection, 2021, ISSN 0163-4453, https://doi.org/10.1016/j.jinf.2021.08.012. (https://www.sciencedirect.com/science/article/pii/S0163445321003947)
5. **Allen, N**., Brady, M., Carrion Martin, A. I., Domegan, L.et al. (2021). SARS-CoV-2 Antibody Testing in Health Care Workers: A Comparison of the Clinical Performance of Three Commercially Available Antibody Assays. *Microbiology spectrum*, *9*(2), e0039121. https://doi.org/10.1128/Spectrum.00391-21
6. **Allen N**, Fleming C, Bergin C (2021). Epi Insight Volume 22, Issue 6 November 2021 Prevalence of COVID-19 in Irish Healthcare Workers (PRECISE 2, April 2021). https://ndsc.newsweaver.ie/4otaa688p3/mu46nnl6o3j?lang=en&a=1&p=60117497&t=31302947
7. **Allen, N**., Brady, M., Ni Riain U et al. (2022). Prevalence of Antibodies to SARS-CoV-2 following natural infection and vaccination in Irish Hospital Healthcare Workers; changing epidemiology as the pandemic progress. *Frontieres in Medicine*, Section Infectious Diseases-Surveillance, Prevention and Treatment. https://doi.org/10.3389/fmed.2021.758118

Presentations

1. Royal Academy of Medicine in Ireland (RAMI) Epidemiology and Public Health Medicine Section, Jacqueline Horgan Bronze Medal Meeting, 13th May 2021 (abstract published in Irish Medical Journal).
2. Infectious Diseases Society of Ireland (IDSI) Annual National Meeting, 2nd June 2021.
3. European Congress for Clinical Microbiology and Infectious Diseases (ECCMID) Conference Oral presentation July 2021 (presented by a co-author on behalf of the study group).
4. Monthly Scientific Meeting – Science Foundation Ireland (SFI) SPP Immunology of COVID-19, 2nd March 2021.
5. St. James's Hospital Immunology Seminar Series, 24th February 2021.
6. European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) – oral presentation November 2021 (presented by a co-author on behalf of the study group).

Awards

1. Royal Academy of Medicine in Ireland (RAMI) Epidemiology and Public Health Medicine Section, Jacqueline Horgan Bronze Medal Winner, 13th May 2021.
2. Infectious Diseases Society of Ireland (IDSI) Annual Conference, 2nd June 2021, Winner of Best Oral Presentation.

Chapter 1: Introduction

1.1 Epidemiology of COVID-19 infection

1.1.1 Global epidemiology of COVID-19 in Healthcare Workers

1.1.2 Epidemiology of COVID-19 infection in Ireland and in Irish healthcare workers

1.2 Laboratory Assays for the detection of SARS-CoV-2 Antibodies

1.3 COVID-19 Vaccination

1.3.1 Vaccines available for COVID-19

1.3.2 COVID-19 Vaccination in Ireland

1.3.3 Antibody Response and Immunity following COVID-19 infection and vaccination

1.4 Description of Study Sites

1.4.1 St. James's Hospital, Dublin

1.4.2 University Hospital Galway

1.4.3 COVID-19 Vaccination at Study Sites

1.5 Research Aims

Chapter 1: Introduction

1.1 Epidemiology of COVID-19 infection

1.1.1 Global epidemiology of COVID-19 in Healthcare Workers

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus was first identified in December 2019 in Wuhan, China, and spread rapidly internationally to cause the most significant pandemic since the Spanish Influenza of 1918, with over 158 million cases worldwide and death toll of 3.3 million by April 2020 (1).

Healthcare workers (HCW), and those that they live with, are at increased risk of contracting COVID-19 viral disease (2) (3) (4) (5) (6). HCW risk acquisition of infection from patients and from each other, and risk passing it to household members and to other staff and patients (7). For multiple reasons, including absence of symptoms, access to PCR testing, stigma, lack of motivation to test, and test sensitivity, only a proportion of total infections in a population will be diagnosed at the time of infection. Seroprevalence studies are useful to estimate the true prevalence of past infection in a population group (8). Although the correlation between antibody positivity and immunity is not yet fully understood for COVID-19, seroprevalence studies can also help to estimate potential immunity in the target population, as well as to identify sub-groups at higher risk of infection acquisition, and groups that should be targeted for vaccination. They can also help to quantify the proportion of asymptomatic infections (8) (9).

SARS-CoV-2 antibody seroprevalence studies have been conducted in HCW in numerous European countries during the first year of the pandemic, and have showed widely varying antibody positivity proportions of between 1% and 45% (10) (11) (12) (13) (14) (15). A large meta-analysis showed an overall seropositivity of 8.5% in European HCW (16). Risk of antibody positivity has been correlated with degree of patient contact (12) (14) (17). Studies have also shown that a significant proportion of infections may be asymptomatic (18). HCW who have asymptomatic infection risk unknowing onwards transmission either in the work or home environment (9) (19).

1.1.2 Epidemiology of COVID-19 infection in Ireland, and in Irish Healthcare workers

Up until the beginning of December 2020, Ireland had had one of the lowest incidence of COVID-19 infection of any European country (1) (20) (21). By the first week of December 2020 Ireland had had just over 75,000 infections, and 2120 deaths, resulting in a cumulative attack rate of 0.015% and a mortality rate of 2.8% (1) (20). From mid-December 2020 the incidence of COVID-19 in Ireland increased dramatically as Ireland headed into its third wave of the pandemic (20). This wave was much larger in magnitude and length than the preceding two waves and resulted in Level 5 restrictions (22) from January 2021 until May 2021 as a mitigation attempt while

vaccination was rolled out. During this time the cumulative incidence rose to over 250,000 infections and the death toll rose to just under 5000 deaths (23).

The Study to investigate COVID-19 Infection in People Living in Ireland (SCOPI) which took place in June/July 2020 tested for antibodies to COVID-19 in members of the community in two areas of Ireland; results showed a national seroprevalence of 1.7%. This was three times higher than the number of PCR-diagnosed infections. Seroprevalence was significantly higher in the Dublin area (3.1%) with comparison to the Sligo area (1.8%) (24). This was in keeping with the difference in incident rates of infection in the two communities during the first wave of the pandemic which preceded the study. Almost three quarters of the population sampled reported having experienced symptoms consistent with COVID-19 viral disease (24).

Prior to this study there were no published literature on the seroprevalence of antibodies to SARS-CoV-2 infection in Irish Healthcare workers (HCW), but it was known from surveillance data that a high proportion of the COVID-19 cases notified were in HCW (25). An unpublished seroprevalence study conducted in Tallaght University Hospital in west Dublin showed that 18% of hospital staff had positive antibodies to COVID-19 (26). Many HCW in Ireland share accommodation, putting them at risk of acquiring or transmitting infection in the home environment as well as the workplace. This is especially true in the Dublin area, where housing is more expensive, and HCW are more likely to share smaller living spaces. Understanding the transmission and immunity dynamics in HCW and in hospitals in Ireland were key factors in controlling the pandemic at national level for multiple reasons including; reduced incidence and onward transmission in the hospital setting, which may also impact positively on community transmission; reduced risk of consequences of infection in HCW, both poor outcomes and staffing issues which directly impact all patient care; reduced risk of transmission of infection from HCW to vulnerable hospitalised patients. These learnings will need to be incorporated into future pandemic preparedness.

1.2 Laboratory Assays for the detection of SARS-CoV-2 Antibodies

Serological assays for the detection of SARS-CoV-2 antibodies have a significant role to play in the response to the COVID-19 pandemic worldwide. Detectable antibody to SARS-CoV-2 is an excellent indicator of past SARS-CoV-2 infection (27) and therefore helps determine the proportion of a population that has been previously exposed or infected. Population seroprevalence studies can provide crucial supplementary information to national surveillance systems and have the benefit of accounting for asymptomatic cases and symptomatic individuals who may not present to the health services for testing. As mass COVID-19 vaccination programmes are rolled out globally, serological assays may have a role in assessing host vaccine response and predicting vaccine effectiveness (28). They may also potentially be used to inform individual risk of disease (29) with emerging evidence of up to six months of immunity after natural infection (30), though further research on this area of post-infection immunity is required.

There are many different commercially available assays for the detection of SARS-CoV-2 antibodies in serum. These include assays that detect certain antibodies only e.g., Immunoglobulin (Ig) G or IgM, as well as panantibody assays which detect more than one antibody. As well as this, antibodies can be directed against different parts of the virus. Two of the commonly detected antibodies are antibodies to the nucleocapsid protein (anti-N) and antibodies to the spike protein (anti-S). Either, or both, of these antibodies can be produced following a natural infection with COVID-19 virus. Anti-spike antibodies are also produced following vaccination against SARS-CoV-2 with the vaccines currently licensed and available for use in Ireland.

Many studies have examined the timing of antibody production and sensitivity of commercially available antibody assays in the early stages of infection with SARS-CoV-2 (31) (32). Few studies have evaluated and compared the longevity of assay sensitivity post PCR-confirmed infection for different assays on the same HCW population. As the pandemic progresses, retained sensitivity over time is an important quality in an antibody assay that is to be used for the purpose of population seroprevalence studies. Decline in SARS-CoV-2 antibody response to natural infection over time has been described (33) (34), and different antibodies may wane at different rates. Some studies have shown sustained antibody detection for up to 100-125 days (35) (36). Understanding the duration of antibody response over time is key to understanding the accuracy of epidemiological studies and informing public health pandemic response measures. Antibodies are easier to measure than other markers of immunity such as T-cell responses, however the extent and duration of immunity and its relationship to antibody positivity is not yet fully understood. The use of serology at individual level for the purpose of estimating immunity also requires detailed understanding of the timing and waning of antibody response in relation to PCR-positivity. A UK study comparing five widely available commercial assays (including the Abbott Architect anti-nucleocapsid IgG assay and Roche Elecsys Anti-SARS-CoV-2 panantibody assay) found them all to have a sensitivity and specificity of at least 98% 30 days post symptom onset (37). Regarding longevity of the antibody response there are few published data, and results differ; a study comparing four different serological assays showed a decline in the performance of the Abbott IgG assay after 60 days, and a further decline after 80 days (38), whereas another study of the same assay showed a mean of 137 days to loss of positive antibody (39). Further data comparing antibody assays for the detection of antibodies to SARS-CoV-2 are needed to guide the most appropriate use of certain commercially available assays in any given situation.

1.3 Variants of Concern

Cases of four variants of concern (VOC) were identified in Ireland between late December 2020 and April 2021; B.1.1.7 (UK1), B.1.351 (South Africa), P.1 (Brazil) and B.1.617.2 (India). The majority of cases sequenced in the third wave of the pandemic in Ireland were due to B.1.1.7. There were very few cases of other VOC amongst the sequenced samples during the time period covered by Precise 1 and 2 (40).

1.4 COVID-19 Vaccination

1.4.1 Vaccines available for COVID-19

By May 2021 there were four COVID-19 vaccines approved by the European Medicines Agency (EMA) for use in human subjects; BNT162b2mRNA - BioNTech (Pfizer), mRNA-1273 (Moderna), ChAdOx1 nCOV-19 (Vaxzevria - formerly COVID-19 Vaccine AstraZeneca) and Johnson & Johnson's Janssen (J&J/Janssen) COVID-19 Vaccine. These vaccines work via different mechanisms. The Pfizer and Moderna vaccines are messenger Ribonucleic Acid (mRNA) vaccines. Vaxzevria and J&J/Janssen are adenoviral-vector vaccines. Pfizer, Moderna and Vaxzevria require two doses to achieve the best immunological response. J&J/Janssen is a single-dose vaccine (41) (42) (43) (44).

1.4.2 COVID-19 Vaccination in Ireland

Vaccination for COVID-19 in Ireland began in late December 2020, with initial roll-out to staff and patients of nursing homes, and to HCW working in acute sector hospitals. The main vaccine available for use in Ireland at the time was the Pfizer vaccine, followed shortly by the Vaxzevria vaccine. From 30th December 2020 until 21st January 2021 HCW were offered the Pfizer vaccine with a dosing interval of 21 days. From 22nd January 2021 until late February 2021 HCW were offered the Pfizer vaccine with a dosing interval of 28 days. From late February 2021 onwards HCW were offered AstraZeneca Vaccine with a dosing interval of 12 weeks. This dosing interval was delayed to 16 weeks in non-urgent cases on the recommendation of the National Immunisation Advisory Committee (NIAC) in April 2021 (45) following global reports of rare thrombosis associated with receipt of AstraZeneca Vaccine (46).

1.4.3 Antibody Response and Immunity following COVID-19 infection and Vaccination

Natural infection with COVID-19 produces both B- and T-cell mediated immunity. There is a degree of waning of this immunity over time: infection-induced immunity has been shown to protect for up to six months (30), while duration of antibody response is varied (35) (38). Vaccines have been shown to be protective both against infection and against symptomatic disease (47). Vaccination is also associated with lower viral loads (48) and decreased duration of oropharyngeal PCR positivity which are very likely to correlate with decreased transmissibility. Vaccine-induced immunity produces a more robust response in both adaptive immune systems, therefore vaccination is likely to protect against infection for a longer duration of time, including against variants of concern (49) (50) . Immunity after natural infection may not protect against re-infection with variants of concern (51) while vaccine-induced immunity is reduced, but not lost, against variants of concern (52) (53). Robust B- and T-cell responses to vaccination have been shown for both mRNA vaccines and viral vector vaccines (54). Antibody response has been shown to correlate with protective immunity against infection (55).

1.5 Description of Study Sites

1.5.1 St. James's Hospital, Dublin

At the peak of the first wave of the COVID-19 pandemic in Ireland, Dublin was the area in Ireland that had the highest number of COVID-19 infections diagnosed. St. James' Hospital (SJH) is a large teaching hospital located in Dublin's south inner city with almost 4,700 employees and over 1000 beds. Nursing staff account for 38% of the workforce, followed by allied health care professionals and laboratory staff (16%), medical and dental staff (14%), administrative staff (14%), health care assistants (10%) and general support staff (8%). Seventy-seven percent of staff on site are female and 48% of staff are over the age of 40 years. Regarding nationality, 77% of staff are Irish, with Indian (7%) and Filipino (7%). From March-May 2020 9.8% (458/4692*) of this workforce tested positive for SARS-CoV-2 infection via PCR, and by the start of October 2020 10.2% of staff had tested positive by PCR, resulting in significant impact on staffing levels in the hospital; 42% of these infections were in nursing staff, 15% in healthcare assistants, 10% in doctors, 5% in health and social care workers, 6% in administration staff and 3% in general support staff (56).

*Denominator data from Human Resources Department, differs slightly from Occupational Health Department denominator data used in published manuscript in *Epidemiology and Infection*. HR data used throughout this thesis for consistency.

1.5.2 University Hospital Galway

University Hospital Galway (UHG) serves as both a regional hospital for Galway as well as being the tertiary referral centre for the SAOLTA hospital group. COVID-19 incident rates in the community in county Galway were significantly lower than that of the greater Dublin area during the first, second and third waves of the COVID-19 pandemic in Ireland (peaks in April and October 2020 and January 2021 respectively (23) (20)) and the regional seroprevalence was assumed to be similar to that of neighbouring Sligo area, where the national seroprevalence study was conducted (24). UHG has over 500 beds and almost 4400 employees, composed of 15% administrative/management staff, 14% health and social care, 39% nursing staff, 5% general support staff, 7% other patient and client care and 20% medical/ dental staff. Seventy-six cases of COVID -19 were diagnosed by PCR and reported to occupational health during the first wave of the pandemic, corresponding to 1.7% (76/4395*) of employees affected; 54% of these infections were in nursing staff and 25% in medical and dental staff. Only 3 further infections were identified up until the start of October 2020; amounting to 1.8% (79/4395) of all employees (57).

The study was planned at the end of the first wave of the pandemic in Ireland and was performed during the second and third waves. The third wave of the pandemic was larger in magnitude and duration nationwide than

the first two waves, and there were an increased proportion of notifications in HCW (58) . The national epidemiology remained similar in these successive wave in terms of highest incidence in the greater Dublin area (23) (20), however the gap in incidence between Dublin and Galway was narrower at certain points of the third wave; in January 2021 the 14-day incidence for Galway approached that of Dublin (59). The community incidence of this third wave peaked in January with a 14-day incidence high of >1400/100,000 cases in Dublin (60). The incidence remaining high through February and March and had a slow tail off into the early summer months. By the start of the second seroprevalence study in April 2021, the incidence was 181/100,000 in Dublin and 83/100,000 in Galway (61).

The third wave impacted both hospitals more significantly than the first and second waves, with multiple hospital outbreaks involving both patients and staff. By the start of April 2021 18.5% (869/4692) of staff of SJH and 9.2% 404/4395) of the staff of UHG had tested positive by PCR. Onsite testing continued at both sites for all staff presenting with symptoms. Asymptomatic testing was carried out in the setting of ward contact or outbreak.

1.5.3 COVID-19 Vaccination at study sites

Both study sites were involved in the initial vaccine roll-out in Ireland in December 2020/January 2021. By the time of the second study in April 2021, most healthcare workers in our study sites who wished to be vaccinated had completed a full course of vaccination. At this time the vaccine uptake rate at each site was unknown, due to centralisation of the vaccination programme. A minority of healthcare workers were still awaiting the second dose of vaccination; this included those who joined the workforce later in 2021, and those who had other delays to first dose vaccination such as a first trimester pregnancy or COVID-19 infection.

1.6 Research Aims

The primary study aim was to perform antibody testing on all healthcare workers in two Irish hospitals to estimate the SARS-CoV-2 seroprevalence amongst a large cohort of HCW working in acute sector hospitals in Ireland. We aimed to compare the prevalence of antibodies to SARS-CoV-2 across hospitals in two geographical areas with higher and lower community and hospital cumulative incidence of COVID-19 in Ireland, as defined at the time of commencing the study. We aimed to estimate the number of HCW that had undiagnosed COVID-19 infection, and to improve understanding of HCW risk factors (demographic, living arrangements and work-place related risks) for SARS-CoV-2 infection, in order to inform health services preparedness for further waves of the pandemic. This study was called PRECISE 1; Prevalence of COVID-19 in Irish Healthcare Workers.

We also aimed to repeat this seroprevalence study six months later, in April 2021, to assess changes in overall seroprevalence at each site as the pandemic progressed, and to describe the changing epidemiology of COVID-19 infection in HCW as the pandemic progressed. This second study (PRECISE 2) had a nested aim of including

longitudinal follow up of linked cases (of HCW who participated in both PRECISE 1 and PRECISE 2) after six months to evaluate antibody decay.

The assay chosen for this seroprevalence study at conception was the Abbott Architect anti-nucleocapsid IgG assay. This was chosen due to its reported high sensitivity and specificity and the Irish experience with this assay during the SCOPI study (24). This also ensured that our results would be directly comparable to the community seroprevalence found by the SCOPI study. During the first seroprevalence study in October 2020 (PRECISE 1), newly published literature suggested that there may be sensitivity issues with the Abbott assay, mostly in relation to increasing time interval between PCR-confirmed infection and serological testing as discussed above (38). We were quickly able to adapt expand our laboratory methodology to include testing on an additional assay to ensure sensitivity of results. We also responded to this emerging field by adding the aim of comparison of antibody detection on these commercially available assays to our study aims. We conducted a secondary analysis with the aim of comparing the prevalence of anti- SARS-CoV-2 antibodies in the same HCW population using two different assays targeting the anti-nucleocapsid protein; Abbott Architect SARS-CoV-2 immunoglobulin (Ig)G assay and Roche Elecsys Anti-SARS-CoV-2 immunoassay (62) (63) (64). We also aimed to compare antibody prevalence for a subset of samples on an assay targeting the anti-spike protein; the Wantai SARS-CoV-2 Antibody ELISA (65).

With the introduction of COVID-19 vaccination, we had to respond again to the rapidly changing landscape of the pandemic, and the initial proposals for PRECISE 2 were revised to include consideration of the roll out of vaccines directed against SARS-CoV-2. The research programme structure was well placed to include assessment of serological and cellular indices of post vaccine immunity (66) (67).

Specific Research Objectives

- To measure the prevalence of antibodies to SARS-CoV-2 in HCW in two hospitals in Ireland in areas of differing COVID-19 incidence
- To report the prevalence of antibodies to SARS-CoV-2 by sex, age group, ethnicity, nationality, area of work, type of patient exposure, previous symptoms, previous PCR testing, and living arrangements
- To examine the relationship between the presence of antibodies to SARS-CoV-2 and the self-reporting of symptoms consistent with COVID-19 or having a previous diagnosis of COVID-19 infection
- To repeat the study after six months to assess changes in overall SARS-CoV-2 sero-prevalence
- To assess changes in individual serostatus for those who participated in both studies separated by six months
- To estimate time to antibody decay for those participants with previously confirmed COVID-19 infection
- To compare commercially available assays for the detection of SARS-CoV-2 antibodies
- To assess indices of immunity and protection post vaccination
- To quantify COVID-19 infections in vaccinated staff

Studies involved and specific aims/ outcomes

1. Cross sectional seroprevalence study of antibodies to SARS-CoV-2 antibodies in HCW, October 2020 (PRECISE 1)
    - To measure the prevalence of antibodies to SARS-CoV-2 in all healthcare workers in two hospitals in Ireland in areas of differing COVID-19 incidence during the first wave of the pandemic.
    - To compare the prevalence of antibodies to SARS-CoV-2 across the two geographical areas with high and low incidence of cases of COVID-19 in Ireland, as defined at the time of commencing the study.
    - To assess the relationship between the presence of antibodies to SARS-CoV-2 and the self-reporting of symptoms consistent with COVID-19 or having a previous diagnosis of COVID-19 infection.
    - To calculate age-specific susceptibility to SARS-CoV-2 infection in the study population.
    - To assess demographic factors relating to SARS-CoV-2 infection in the study population.
    - To compare antibody prevalence by working role, degree of patient exposure, and living arrangements.
    - To quantify asymptomatic infections.
    - To quantify undiagnosed infections.

2. Cross sectional seroprevalence study of antibodies to SARS-CoV-2 antibodies in HCW, April 2021 (PRECISE 2)
    - To measure the prevalence of antibodies to SARS-CoV-2 in all healthcare workers in two hospitals in Ireland in areas of differing COVID-19 incidence in the first, second and third waves of the pandemic.
    - To compare the prevalence of antibodies to SARS-C0V-2 across the two geographical areas with high and low incidence of cases of COVID-19 in Ireland.
    - To compare the prevalence of antibodies to SARS-CoV-2 in HCW at the same location at two points in time separated by 6 months.
    - To compare the seroprevalence in HCW after the first wave of the pandemic in Ireland versus after the second and third waves of the pandemic in Ireland (PRECISE at the start of the second wave; PRECISE 2 in the decline of the third wave).
    - To assess the relationship between the presence of antibodies to SARS-CoV-2 and the self-reporting of symptoms consistent with COVID-19 or having a previous diagnosis of COVID-19 infection.
    - To calculate age-specific susceptibility to SARS-CoV-2 infection in the study population
    - To assess demographic factors associated with antibody positivity in the study population.

- To compare antibody prevalence by working role, degree of patient exposure, and living arrangements.
- To quantify asymptomatic infections.
- To quantify undiagnosed infections.

3. Comparison of Commercially available Antibody Assays for the detection of SARS-CoV-2 antibodies in HCW
    - Difference in overall seroprevalence in study population; comparison on two assays.
    - To compare the performance of three assays in participants with previous PCR- confirmed SARS-CoV-2 infection.
    - To compare positivity and concordance for all samples on two assays.
    - To conduct a sub-analysis of sample results within manufactures' suggested grayzone.
    - To compare positivity and concordance for the subgroup of those will previously PCR-confirmed infection.
    - To compare positivity and assay concordance by time point in the previously PCR-confirmed subgroup.
    - To describe the subset of participants who were previously PCR-positive but who had no detectable antibody by serology (i.e. discordant results).
    - To measure the distance to positive threshold of these discordant results.

4. Study to evaluate the decay in SARS-CoV-2 antibody levels over a six-month time period
    - To conduct a longitudinal follow up of participants common to PRECISE 1 and PRECISE 2.
    - To assess changes in antibody status related to self-reported PCR-confirmed infection, date of infection, severity of symptoms, and quantitative antibody result.
    - To assess waning of antibody over time as a surrogate for duration of potential immunity post natural infection.

5. Cross-sectional Study of antibody response to COVID-19 vaccination
    - To measure of anti-spike protein to assess antibody production post vaccination.
    - To study the relationship between anti-spike antibody result and date of vaccination(s), quantity of vaccinations (i.e., fully or partially vaccinated), vaccine type and dosing interval.
    - To quantify the number of PCR-confirmed infections post vaccination and date of occurrence with relation to timing of vaccination.
    - To assess the severity of symptoms in those with confirmed infection post vaccination.

1.7 Hypothesis

Healthcare workers in Irish hospitals are at increased risk of acquisition of COVID-19 infection. Transmission of COVID-19 is occurring from HCW to HCW, and between HCW and patients. HCW also transmit and acquire COVID-19 infection in the household, therefore hospital transmission dynamics are strongly linked to community transmission dynamics. Our hypothesis was extended with the advent of vaccination to include that a large proportion of fully vaccinated individuals develop anti-spike SARS-CoV-2 antibodies.

- A higher seroprevalence in St. James' Hospital compared to University Hospital Galway, reflecting community transmission.
- A higher seroprevalence in HCW performing roles with close patient contact.
- A proportion of HCW with previously undiagnosed infection.
- A proportion of asymptomatic infections.
  (There was no pre-existing evidence to guide prediction of what these proportions might be)
- An increase in seroprevalence among HCW from October 2020 to April 2021.
- Comparable performance of different antibody assays for the detection of SARS-CoV-2 antibodies, based on manufacturers' stated sensitivities.
- Waning of antibody levels over a six-month time period.
- Anti-spike antibody positivity in the majority of fully vaccinated HCW, regardless of vaccine brand.

Chapter 2: Methods

2.1 Research Inception and Design

2.2 National Ethical Approval and Funding

2.3 Participants and Procedures

2.3.1 Inclusion Criteria

2.3.2 Communication strategy

2.3.3 Use of Information Technology Platforms

2.3.4 Results management and dissemination

2.4 Sample Testing and Laboratory Methods

2.5 Statistical Methods

2.6 Data Matching and Database Management

Chapter 2: Methods

2.1 Research Inception and Design

This research was designed by me and the lead site investigator at each research site, in the summer of 2020, following the first wave of the COVID-19 pandemic in Ireland. The research was guided by a multidisciplinary research steering committee and endorsed by the National Public Health Emergency Team (NPHET). At that point in time, a seroprevalence study was underway in the Irish community population (24), but there was no data on the SARS-CoV-2 seroprevalence amongst Irish HCW, and this was deemed a priority in order to control the pandemic at national level. Early international data had shown increased rates of infection among HCW, and the risks of onwards transmission to patients, other HCW and to their families, but transmission dynamics varied depending on location, making data difficult to generalise to our setting (3) (7) (8) (10) (14). We needed to understand transmission dynamics in the Irish hospital setting, both in areas of high and low community incidence, as national IPC guidelines were being developed and constantly revised to reflect the dynamic early pandemic situation. We needed to improve understanding of HCW infections and in-hospital transmission in order to decrease the number and magnitude of hospital outbreaks, and the associated morbidity and mortality among staff and vulnerable patients, and to increase understanding of the number of HCW that may be unknowingly infected and transmitting COVID-19 infection in the household.

Two Irish hospitals were selected, as discussed in the introduction, and a study steering committee was set up consisting of public health experts from the Health Service Executive Health Protection Surveillance Centre (HSE/HPSC), representatives of the Infectious Diseases, Microbiology, Immunology and Occupational Health Departments of both hospitals, and virology experts from the National Virus Reference Laboratory (NVRL). I was invited to be the Principal Investigator for the research programme.

The initial research design was a longitudinal seroprevalence study, consisting of two seroprevalence -surveys occurring six months apart, in October 2020 and in April 2021. The two cross-sectional studies (PRECISE 1; Chapter 3 and PRECISE 2; Chapter 5) were carried out from 14th -23rd October 2020 and the 19th- 28th April 2021 respectively. The sub-study on changes in individual serostatus over a six-month time-period (Chapter 6) was a longitudinal study of linked data from PRECISE 1 and PRECISE 2. Two further sub-studies arose as we responded to the dynamic nature of a pandemic; the study of comparison of laboratory assays for the detection of antibodies to SARS-CoV-2 (Chapter 4) was conducted using additional data collected during PRECISE 1, as a response to evolving data on sensitivity over time with certain commercially available laboratory assays for the detection of SARS-CoV-2 antibodies; the study on antibody response to COVID-19 vaccination (Chapter 5) was added to the initial plans for PRECISE 2 with the roll-out of vaccination in Ireland, and involved planning for additional testing of all samples on a new anti-spike assay that had not yet been validated in either hospital.

2.2 National Ethical Approval and Funding

I obtained ethical approval from the National Research Ethics Committee (NREC) for COVID-19, Study Number 20-NREC COV-101 (33). Amendments to the study testing methodology were requested by me and granted in January 2021 by NREC, to include testing for serological response to vaccination. I lead the team to develop a detailed budget for each study and funding to the value of e665,116 was secured through applications to the Health Service Executive (HSE) COVID-19 budget.

2.3 Participants and Processes

2.3.1 Inclusion Criteria

All staff members of both hospitals were invited to participate, regardless of area of work. Participation was via an online self-administered consent and questionnaire (68) followed by a blood test on site at their location of work. Inclusion criteria was all staff members of both hospitals regardless of area of work. This comprised 4692 HCW in SJH and 4395 HCW in UHG, making up a total of 9038 HCW invited to participate. Those who were in quarantine or self-isolating during the dates of the blood sampling were excluded from participation. Students on attachment in the hospitals were also excluded from participation.

Sample size calculation

The target population was all staff of both hospitals; 4692 (SJH) and 4395 (UHG). We anticipated an uptake of 70-80% based on other similar studies of COVID seroprevalence in healthcare workers (13) (10). This amounted to 3284 - 3754 staff in SJH and 3076-3516 in UHG. Considering a pessimistic non-response rate of 30%, we would have been able to include 1,407 staff in SJH and 1,318 staff in UHG. Considering an extreme (and conservative for sample size calculation purposes) scenario of 20% we would have a precision that would range from 1-2%.

2.3.2 Communication Strategy

For information about PRECISE 1 was disseminated from September 2020 onwards, via all-staff emails, text messages, hospital intranet, hospital meetings, heads of departments and discipline champions. Concerted efforts were made to directly target groups that are traditionally harder to access, who may not engage with hospital IT messaging services. Due to the online nature of the participant questionnaire, I was able to track the daily uptake and bookings both quantitatively and qualitatively, and adjust the hospital and individual group messaging accordingly, in order to target the disciplines with reduced uptake. Along with the study team, I organised specific meetings with domestic staff, portering staff, security staff and HCAs in order to inform them of the study and help them with the online component if required. Our staff members are diverse ethnically and speak many different languages. To overcome any potential language barrier all study information were available in the five most spoken languages in the hospitals (English, Filipino, Portuguese, Indian and Polish). I designed the

study website, which contained information in all five languages, and hard copy posters and participant information leaflets were disseminated widely in the hospital in all five languages. This methodology was repeated in April 2021 for PRECISE 2. The messaging for PRECISE 2 included the fact that the study would now also measure antibody response to vaccination. In the leadup to both PRECISE 1 and PRECISE 2, the country was under level 5 restrictions (22), which made any group gatherings difficult. This meant that all messaging had to be done in smaller group environments, with no hospital-wide face-to-face meetings taking place. This increased the challenge to secure good uptake for the study.

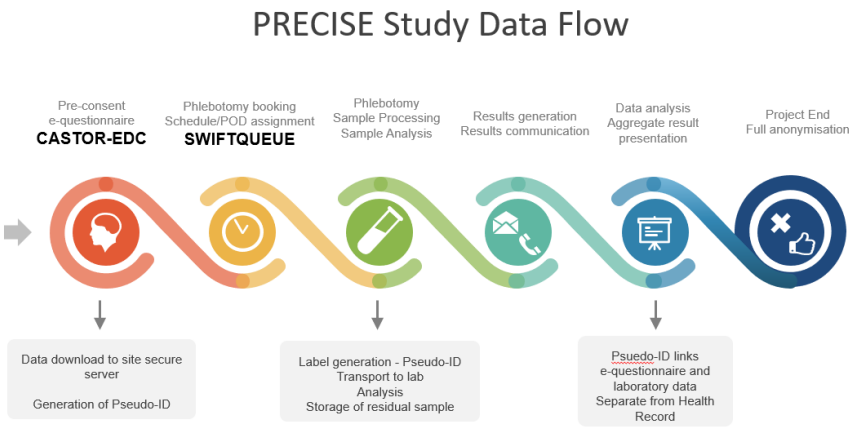
2.3.3 Use of Information Technology Platforms

The online consent platform and questionnaire opened for participation three weeks prior to the commencement of blood sampling for each serosurvey. Blood sampling took place simultaneously in both hospitals, from the 14th - 23rd October and from the 19th-28th April 2021. Following online consent, in October participants were asked for information about their demographics, role in the workplace, type of patient contact and whether they were also working in the hospital or any other nursing home from January -June 2020. Participants were defined as high risk if reporting daily contact with patients with known or suspected COVID-19 infection, intermediate-risk if reporting daily contact with patients without known or suspected COVID-19 infection and low-risk if reporting little or no patient contact. They were asked about personal previous laboratory confirmed infection, symptoms, close contact with a confirmed case of COVID-19 and previous testing because of this contact or for any other reason. The questionnaire also elicited details of living arrangements; number of people in the household, children versus adults, sick household contacts, and whether they lived with other HCW.

The repeat serosurvey in April 2021 was conducted in a similar manner. The online questionnaire was adjusted to facilitate those who had participated in October 2020 not having to repeat their contact and testing details. Amendment to the ethical approval was also obtained for the addition of questions pertaining to vaccination history; specifically, the type of vaccine received, the number and date of doses received, and whether the participant had had PCR-confirmed COVID-19 infection post-dating vaccination. In April 2021 participants were also asked for consent to access their vaccination records, if necessary, to confirm dates or types of vaccine, and were asked for consent to link their data from PRECISE 1 and PRECISE 2 if they participated in both.

Following completion of the questionnaire, I designed the system to automatically transfer a participant to the online booking system for the blood test (69). The participants would be directed to the booking system landing page appropriate to their working location. Following selection of a timeslot for their blood test, the participant received a confirmation text message and/or email of their booking time. On arrival for phlebotomy, the phlebotomist selected this booking, which lead to the automatic generation of a random unique participant identifier code (MRN – medical record number) for each participant. This unique MRN and the participant's date of birth were the only information placed on the sample to ensure anonymous processing in the laboratory.

Figure 1: PRECISE Study Data Flow and use of IT systems



To the best of our knowledge, this is the first study of its kind in Ireland to be conducted using a fully online consent process and an online questionnaire.

2.3.4 Results management and dissemination

Participants were asked in the questionnaire whether they would like to receive the results of their antibody test. For PRECISE 1, results were issued by text message to those in whom antibodies were not detected. Those with a detected result were phoned by the study team to discuss the meaning of the result. A phone line was established for those with a not detected result who wished to discuss their result. All participants were offered a virtual clinic appointment if they wished to discuss their results with a doctor, and I coordinated a team of doctors to provide this service. For PRECISE 2, all results were issued by text message. Participants who wished to discuss their result could phone or email the study team to discuss the result, or to book a virtual clinic appointment with a doctor if they wished to discuss specifically with a medical professional.

The issuing of personal results was one of the factors that was associated with the high uptake of the study. This aspect of the study required detailed deliberation over wording and message communication to staff due to the constantly evolving field of understanding of COVID-19 antibody response and immunity. We had to ensure that the study did not cause either unnecessary worry to HCW, nor a relaxation or perceived exemption from public health recommendations due to real or perceived immunity. We had to stress the nature of research to inform, as opposed to clinical testing. This was especially true for the results management for PRECISE 2, where a number of results combinations were possible; participants vaccinated or not, with none, one or both antibodies detected. The study team and the study steering group discussed in detail the approach to the results delivery, especially for the scenario of participants who were vaccinated yet did not have antibodies detected; at the time of the study the exact relationship between antibody positivity and immunity was not clear, and there were no clinical guidelines for this scenario (change to working role, repeat vaccination etc) as serological assessment post vaccination was not routinely indicated for any scenario. For all of these reasons, constant engagement with staff

on a group and personal level was required throughout the study period to ensure they accompanied the study team on the research journey.

2.4 Sample testing and Laboratory Methods

For PRECISE 1, samples were initially tested with the Abbott Architect SARS-CoV-2 immunoglobulin (Ig) G assay, directed against nucleocapsid (anti-N) protein (70), as used for the SCOPI Study of community antibody prevalence in Ireland (24). An index value of ≥1.40 Sample/Calibrator (S/C) was considered positive, based on the manufacturers threshold for seropositivity at the time of testing (62) (63). Samples with an index value of 0.5-1.39 S/C were referred for additional testing, in line with the manufacturers' suggested grayzone at the time of testing (7). All positive and grayzone samples were sent to the National Virus Reference Laboratory (NVRL) for additional testing with the Wantai SARS-CoV-2 Antibody ELISA distributed by Fortress Diagnostics, which targets anti-spike (anti-S) antibodies. During the testing process, newly published literature highlighted a potential decline in detection of antibodies with the Abbott Architect SARS-CoV-2 immunoglobulin (Ig) G assay after 60 days (38) (71). In response to this, in addition to having implemented additional testing for samples in Abbott's updated grayzone to improve assay sensitivity, we decided to also test all samples with the Roche Elecsys anti-SARS-CoV-2 total antibody immunoassay, also measuring antibodies to nucleocapsid protein (anti-N), including IgG, to ensure adequate sensitivity based on evolving understanding of the sensitivity of various assays over time. A cut-off index (COI) of ≥1.0 in the Roche Elecsys anti-SARS-CoV-2 assay was considered positive, based on the manufacturer's threshold. (64). To prioritise sensitivity, a positive result on any of the three assays (Abbot, Roche or Wantai) was considered a positive result and qualitatively reported as antibody detected.

In April 2021, for PRECISE 2, the laboratory methodology was changed slightly in order to reflect the evolving situation, including the national roll-out of vaccination to all HCW. Following research and cost-assessment, we used the Roche Elecsys assay to test for both anti-N and anti-S antibodies, to be able to differentiate between antibody response to natural infection and antibody response to vaccination (64) (72).

The assays used on site in the hospitals (Abbott anti-N IgG assay, Roche anti-N panantibody assay and Roche anti-S panantibody assay) required validation prior to their use in the study. This process was performed by the laboratory staff. The Wantai anti-spike assay, performed in the NVRL, was already in use in the NVRL at the time of commencing the study.

2.5 Data matching and database management

The study data was derived from three sources - the laboratory results, the online questionnaire platform and the online blood booking system. These three sets of data had to be matched in order to identify participants, link them to their results via their study MRN, and then further link them to their study questionnaire using initials and date of birth (DOB). For this step of the data matching, first, duplicate initials and dates of birth

combinations were excluded, and all others were matched using statistical software. Then those with duplicate initials and DOB were matched individually. Once one final database had been constructed, we extracted lists of participants according to their results and whether or not they wished to receive results. These were uploaded into an online platform to send secure web-based text messages containing results. Those participants whose result required a phone call were extracted into a separate database.

I organised the data flow (Figure 1) and coded the data for data processing and analysis. The databases were stored by the data manager onsite in each hospital, under my supervision.

2.6 Statistical analysis

Data management and statistical analysis were performed using Microsoft Excel 2016, Stata version 16 and R version 4.0.3 statistical software. Details of specific analysis performed are described in the relevant chapters.

Chapter 3: Seroprevalence of antibodies to SARS-CoV-2 in Hospital Healthcare Workers, October 2020; a cross-sectional study

Chapter 3: Seroprevalence of antibodies to SARS-CoV-2 in Hospital Healthcare Workers, October 2020; a cross-sectional study**

**see copy of published scientific manuscript in Appendix 2

3.1 Introduction

Hospital healthcare workers (HCW) are at increased risk of contracting COVID-19 infection (2) (5). The community seroprevalence of SARS-CoV-2 antibodies in Ireland was found to be 3.1% in Dublin and 1.8% in Sligo following the first wave of the pandemic in Ireland (24). The seroprevalence amongst Irish HCW was not known. Understanding HCW transmission dynamics in the healthcare setting was a priority in controlling the epidemic at national level. We aimed to determine the seroprevalence of SARS-CoV-2 antibodies in HCW in Ireland. Two tertiary referral hospitals in Irish cities with diverging community incidence and seroprevalence were identified as detailed in Chapter 1; COVID-19 had been diagnosed in 10.2% (458/4692) of the staff of St. James's Hospital and 1.7% (76/4395) of the staff of University Hospital Galway by the time of the study (October 2020).

3.2 Methods

All staff of both hospitals (N=9038) were invited to participate in an online questionnaire and blood sampling for SARS-CoV-2 antibody testing, as described in detail in Chapter 2. Information was collected on demographics, living arrangements, working role, degree of patient contact, previous close contact with a confirmed case of COVID-19 (at work or in the community), previous symptoms consistent with COVID-19 infection and past testing for or confirmed diagnosis of COVID-19 infection.

3.2.1 Laboratory Methods

Participant samples were tested on-site in each hospital, and a subset of samples underwent additional testing in the NVRL, as detailed in Chapter 2.4. All samples were tested on two serological assays; Abbott IgG antibody assay (62) and Roche Elecsys panantibody assay (72), both testing for anti-nucleocapsid antibodies. Samples with results falling in the Abbott manufacturers' positive or grayzone underwent additional testing in the NVRL on the Wantai ELISA total antibody assay (65), which detects antibodies to the spike protein. In order to maximise sensitivity, a detectable antibody on any of the three assays was considered a positive result.

3.2.2 Statistical Methods

Frequencies and percentages were calculated for sociodemographic, epidemiological and clinical characteristics, including antibody results. Characteristics of those with a positive SARS-CoV-2 antibody result were compared to those with undetectable antibody, using the chi-square test. Univariate logistic regression was used to calculate relative risks (RR) along with their 95% confidence intervals to assess the association between detected SARS-

CoV-2 antibody and characteristics of the study participants. Multivariable regression analysis was conducted to control for negative and positive confounding and to estimate adjusted relative risks (aRR) for key explanatory variables identified during the univariate analysis. Relevant variables reporting p-value <0.2 were considered for inclusion in the multivariable model. Forward stepwise variable selection was used, and the best fitting and most parsimonious model was selected based on the Akaike information criterion. Post-stratification weighting was explored to calculate overall seroprevalence and to account for different response rates in different HCW roles groups. For the sake of parsimony, we report only unweighted results considering that we found no significant difference from the weighted versus un-weighted seroprevalence. Data management and statistical analysis was performed using Stata version 16 and R version 4.0.3 statistical software.

3.3 Results

3.3.1 Participation rates and demographics

All staff working in SJH and UHG (9,038 people) were invited to participate in the study. In total 5921 blood samples were collected representing a 66% (3115/4692) uptake in SJH and a 64% (2806/4395) uptake in UHG. Of all samples collected, 97% (5788/5921) of samples had a matching questionnaire completed, representing 65% (3042/4692) participation rate for both questionnaire and blood test in SJH and 63% (2745/4395) in UHG.

The socio-demographic characteristics of participants were similar in both hospitals. Seventy-seven percent were female, median age was 39.5 (IQR 30.4-48.9); 5.1% of participants were >60 years of age; 77% of participants were white Irish, 10% Asian (13% in SJH, 7% in UHG, p<.001), 9.5% were other white background (majority born in Poland, USA, and UK) and 2% were of African or any other black background. Eighty-eight percent of participants had third-level or postgraduate education; 91% of participants lived with others, and 31% lived with other HCW. The majority (36%) of participants were nursing staff, followed by allied health care staff (19%), medical/dental staff (17%), administration staff (13%), general support staff (7.5%), health care assistants (HCA) (5%) and other (2%), broadly reflecting the HCW breakdown of the hospital staff (Table 1a). Participation rates among staff groupings were similar in both hospitals; nurses and health care assistants were slightly under-represented at 59% and 39% uptake respectively. In all other groups participation rate was above 60% (for detailed figures on participation see Table A-D, Annex).

Table 1a. Participant characteristics by hospital and total number of participants

Participant characteristics		St James's Hospital (N=3,042)		University Hospital Galway (N=2,745)		P-value*	Total (n=5,788)	
		n	%	n	%		N	%
Age groups	18-29	728	24	632	23	0.717	1,350	23
	30-39	831	27	785	29		1,617	28
	40-49	793	26	722	26		1,515	26
	50-59	532	18	468	17		1001	17
	Over 60	158	5.2	146	5.3		304	5.3
Sex	Female	2,326	77	2,152	78	0.117	4,478	77
	Male	716	24	592	22		1,308	23
	Other	-	-	-	-		-	-
	Missing	-	-	1	0.04		1	0.02
Ethnicity	Irish	2,262	74	2,182	80	<0.001	4,444	77
	Any other white background	267	8.8	284	10		551	10
	Any Asian background	393	13	184	7		577	10
	Any African or black background	65	2.1	48	1.8		113	2.0
	Other	55	1.8	46	1.7		101	1.8
	Missing	-	-	1	0.04		1	0.02
Country of birth*	Ireland	2,182	72	2,091	76	<0.001	4,273	74
	United Kingdom	152	5.0	192	7.0		344	5.9
	India	201	6.6	98	3.6		299	5.2
	Philippines	166	5.5	25	0.9		191	3.3
	Poland	24	0.8	48	1.8		72	1.2
	USA	22	0.7	38	1.4		60	1.0
	Other	295	9.7	253	9.2		548	9.5
	Missing	-	-	-	-		-	-
Education	Primary	27	0.9	2	0.1	<0.001	29	0.5
	Secondary	420	14	264	10		684	12
	Third level	1,300	43	1,245	45		2,545	44
	Post-graduate	1,295	43	1,232	45		2,527	44
	Missing	-	-	2	0.1		2	0.03
Role	Admin	454	15	349	13	<0.001	803	14
	Medical/dental	460	15	522	19		982	17
	Nursing/ midwifery	1045	34	1,019	37		2064	36
	Allied health	616	20	475	17		1012	19
	General support	255	8.4	179	6.5		434	7.5
	Health care assistant	157	5.2	129	4.7		286	4.9
	Other	55	1.8	72	2.6		127	2.2
	Missing	-	-	-	-		-	-
Lives with	Alone	256	8.4	223	8.1	0.020	479	8.3
	With others	2,768	91.0	2,518	91.7		5,286	91.3
	Missing	18	0.6	4	0.2		22	0.4
Lives with HCW	Yes	928	31	839	31	0.983	1,767	31
	No	2,060	68	1,859	68		3,919	68
	Missing	54	1.8	47	1.7		101	1.8

* Calculated using the Chi-Square test

Previous exposure, symptoms and testing

In terms of previous exposure, 39% of participants in SJH had previously been a close contact of a confirmed case of COVID-19 infection, compared to only 19% of those in UHG. In both hospitals, 87% of these reported close contact events occurred at work. Seventeen percent of participants in SJH reported daily contact with confirmed cases of COVID-19 as part of their work; this was 14% of participants in UHG. A further 53% of the participating staff of SJH reported daily contact with patients without suspected or confirmed infection with COVID-19, compared to 60% in UHG. And 30% in SJH, 26% in UHG had little or no patient contact (Table 1b).

Symptoms consistent with COVID-19 had occurred in 55% of SJH staff and 45% of UHG staff, for a total of 50% of participants experiencing symptoms (in both hospitals) at some stage; 35% of these were minor symptoms (equal to a cold or less), 9.1% had significant symptoms (comparable to a bad flu, bed-ridden) and 0.4% were hospitalised. These figures were similar in both sites (Table 1b).

In terms of self-reported previous testing, 55% of SJH staff and 40% of UHG staff had previously undergone PCR testing for infection with COVID-19. In SJH 9.6% of participants reported having previously tested positive by PCR compared to 2.7% of UHG participants (Table 1b).

Table 1b. COVID-19 related characteristics by hospital and total number of participants

Participant characteristics		St James's Hospital (N=3,042)		University Hospital Galway (N=2,745)		P -value*	Total (N=5,788)	
		n	%	n	%		n	%
Contact of a COVID-19 case	Yes	1,185	39	519	19	<0.001	1,704	30
	No	1,847	61	2,224	81		4,071	70
	Missing	10	0.3	2	0.1		12	0.2
Setting of close contact	Contact at work	1,039	88	456	88	0.916	1,495	88
	Contact outside of work	146	12	63	12		209	12
	Missing	-	-	-	-		-	-
Daily contact with COVID-19 patients	Contact with COVID-19 patients	510	17	392	14	<0.001	902	16
	Contact with patients without COVID-19	1,611	53	1,634	60		3,245	56
	No patient contact	918	30	717	26		1,635	28
	Missing	3	0.1	2	0.1		5	0.1
Previous COVID-19 symptoms	No symptoms	1,359	45	1,517	55	<0.001	2,876	50
	Had symptoms	1,683	55	1,228	45		2,911	50
Severity	No symptoms	1,359	45	1,517	55	<0.001	2,876	54
	Only minor symptoms	1,105	36	915	33		2,020	35
	Significant symptoms	298	9.8	230	8.4		528	9.1
	Had symptoms but their severity is unknown	270	8.9	70	2.6		340	5.9
	Hospitalised	10	0.3	13	0.5		23	0.4
Previous COVID-19 PCR test	Yes	1,685	55	1,093	40	<0.001	2,778	48
	No	1,353	45	1,650	60		3,003	52
	Missing	4	0.1	2	0.1		6	0.1
Previous positive COVID-19 PCR test	Yes	292	9.6	75	2.7	<0.001	367	6.3
	No	2,746	90.3	2,668	97.2		5,414	93.6
	Missing	4	0.1	2	0.1		6	0.1

3.3.2 Overall SARS-CoV-2 seroprevalence by location and working role

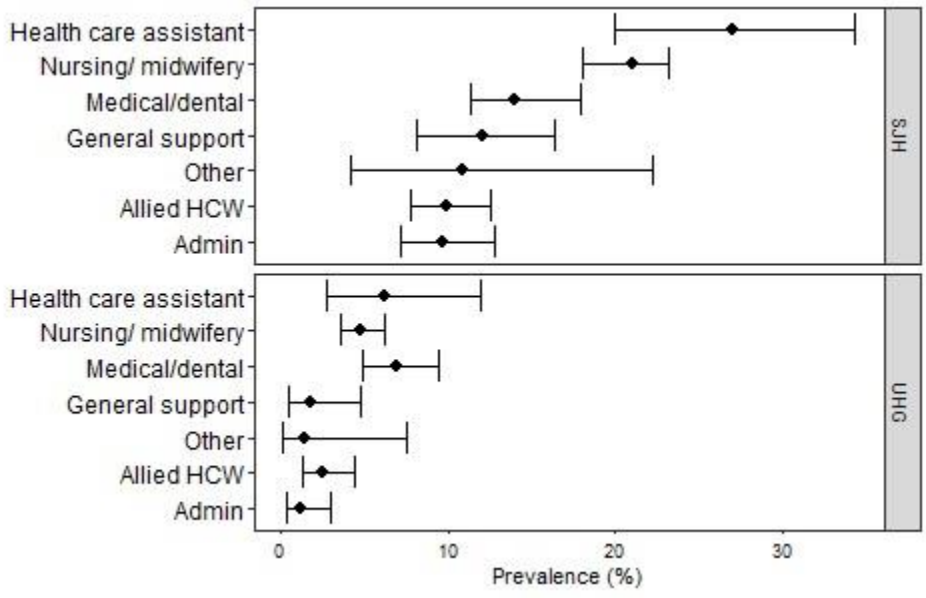
In SJH, the overall SARS-CoV-2 seroprevalence was 15% (464/3042). It rose to 21% (108/510) in those who reported daily contact with known or suspected cases of COVID-19, 17% (269/1611) in those who reported daily contact with patients without known or suspected COVID-19 infection, and 9.5% (87/918) in those who reported little or no patient contact (Table 2d, appendix). Seroprevalence by professional subgroup was highest in HCAs

at 27%, followed by nurses (21%), medical/ dental professionals (14%), general support staff (12%), allied healthcare professionals (11%) and administrative staff (9.5%), but confidence intervals overlap (Figure 2) (Table E Annex).

In UHG, the overall seroprevalence was 4.1%. It was 7.1% (28/392) in those who reported daily contact with known or suspected cases of COVID-19, 4.6% (75/1634) in those who reported daily contact with patients without known or suspected COVID-19 infection, and 1.3% (9/717) in those who reported little or no patient contact (Table 2f, appendix). Seroprevalence by professional subgroup was highest in medical/dental staff at 6.9 %, followed closely by HCAs (6.2%) and nurses (4.7%), but confidence intervals overlap (Figure 2) (Table F, Annex).

The combined data for both hospitals showed that HCAs were significantly more likely to be antibody positive with 18 % of those participating in the study having detectable antibodies. This was followed by nurses at 13% and medical/dental staff at 10%**.** The group with the lowest seroprevalence were the administration staff at 6% (Table G Annex).

Figure 2. Proportion of staff group with detectable antibodies to SARS-CoV-2, both hospitals, October 2020



3.3.3 SARS-CoV-2 Seroprevalence by history of previous PCR testing and symptoms

Ninety-five percent of those who reported a previous positive PCR had detectable antibody (350/367, 95.4%). The majority of those with previous positive PCR (358/367, 97.6%) had been symptomatic at the time of the positive PCR. There was no statistically significant difference between the proportion of antibody positive between those who were symptomatic at the time of positive PCR, 95.6% (325/340), and those who were not symptomatic 92.0% (23/25).

Sixteen percent (90/576) of the participants who had a detectable antibody reported never having had symptoms consistent with COVID-19 infection at any stage (Table H, annex).

Eight-hundred participants reported that they had experienced symptoms at some stage but had never been tested by PCR for COVID-19 infection. Forty-three of these participants (43/800, 5%) had a detectable antibody, meaning that these people possibly continued to work while having symptoms and not seeking a test for COVID-19. Nine of these participants who had never been tested and had detectable antibody reported significant COVID-19 like symptoms at some stage.

3.3.4 Previously Undiagnosed COVID-19 Infection

In total, 576 participants (464 in SJH and 112 in UHG) had detectable antibodies. Of these, 226/576 (39%) had never been diagnosed with COVID-19 infection. This represented at least 3.9% of the total study population having a previously undiagnosed infection. The majority of those who had never been diagnosed (141/226 (62%)) had experienced COVID-19 like symptoms at some stage, and 37/141 (26%) of those with symptoms reported that they had experienced significant symptoms. Despite this, 101/226 of these participants who were antibody positive but had never been diagnosed had never undergone PCR testing at any stage. The majority of these undiagnosed infections were in SJH employees (187/226, 83%), and most (169/187, 90%) had worked in SJH throughout the first wave of the pandemic. Most of the participants with undiagnosed infections reported daily patient contact in their role (188/226, 83%); 89/226 (40%) were nurses and 33/226 (14%) were doctors.

3.3.5 Relative Risk of Antibody Positivity

Characteristics of those participants who were antibody positive compared with those who were antibody negative for both hospitals combined are shown in Tables 2. Those of male sex, and those in the 18-29-year age

group had a higher seroprevalence; 12% of all participating males had detectable antibody versus 9.4% of females (p=.013), and 13% of all participants aged 18-29 had detectable antibodies (p<.001). Regarding ethnicity 19% of Asian participants and 14% of participants of African or other black background were seropositive, versus 8.6% of white Irish participants (p<.001). Detectable antibody was found in 18% of HCAs, 13% of nurses, 10% of doctors, 7.6% of general support staff, 6.7% of allied health professionals and 6.0% of administration staff (p<.001). Ten percent of those living with others had detectable antibody compared to 5.9% of those living alone (p=.007), and 13% of those living with other HCW had detectable antibodies compared with 8.5% of those not living with HCW (p<.001). Those with daily exposure to patients with confirmed or suspected COVID-19 infection had a seropositivity of 15%, compared to 11% in those with daily contact with patients without confirmed or suspected COVID-19 infection, and 5.9% of those with little or no patient contact (p<.001). Nineteen percent of those who reported at least one close contact with a confirmed case had detectable antibody, compared to 6.1% of those who had never had a close contact event (p<.001); 27% of those in whom the contact was in the community or household had detectable antibody, versus 18% of those in whom the close contact event was in the workplace (p=.002) (Table 2a and 2b).

The characteristics of those participants who were antibody positive in each hospital are shown in Tables 2c-2f (annex). The main differences in these characteristics between the two hospitals were in sex and working role. There was a strong association with being antibody positive if male in UHG (6.3% of males versus 3.5% of females seropositive, p<.003), but the difference between seropositivity in males and females in SJH was less pronounced (Tables 2c and 2e, annex). The differences in breakdown by working role is described above. Regarding living arrangements, in SJH the association between living arrangements and seropositivity was much stronger than in UHG; SJH participants living with others had double the seroprevalence than those living alone (16% versus 8%, p<.004) and 21% of those living with other HCW were antibody positive compared to 13% of those living with non-HCW, p<.001). The data for UHG shows a similar trend, but with a lower overall prevalence (Tables 2d and 2f, annex).

On multivariable analysis of the combined hospital data, the adjusted relative risk of detectable antibody was higher for the following characteristics: working in SJH (aRR 3.7, 95% CI 3.0-4.5. p<.001), being a healthcare assistant (aRR 2.0, 95% CI 1.4 – 3.0, p 0.001), nurse (aRR 1.6, 95% CI 1.1 – 2.2, p 0.007), daily exposure to patients with confirmed or suspected COVID-19 infection (aRR 1.6, 95% CI 1.2-2.1, p 0.002), daily contact with patients not known or suspected to have COVID-19 infection (aRR 1.4, 95% CI 1.1-1.8, p 0.008), age 18-29 (aRR 1.4, 95% CI 1.1-1.9, p 0.006), living with others (aRR 1.5, 95% CI 1.0-2.1, p 0.048), living with other HCW (aRR 1.3, 95% CI 1.1 – 1.5, p 0.007), being of Asian background (aRR 1.3, 95% CI 1.0-1.6, p 0.028) and male sex (aRR 1.2, 95% CI 1.0-1.4, p = 0.046). (Table 3).

On multivariable analysis by hospital, in SJH the aRR of detectable antibody was statistically significant for the following characteristics: being a HCA (aRR 1.0, 95% CI 1.3-3.0, p=.002), being a nurse (aRR 1.6, 95% CI 1.1-2.2, p=0.013), living with others (aRR 1.6, 95% CI 1.0-2.4, p=.037), living with other HCW (aRR 1.3, 95% CI

1.1-1.6, p= 0.003), daily contact with patients with known or suspected COVID-19 infection (aRR 1.4, 95% CI 1.0-1.9, p=0.036), daily contact with patients without known or suspected COVID-19 infection (aRR 1.3, 95% CI 1.0-1.7, p=0.0398), and being aged 18-29 (aRR 1.3, 95% CI 1.0-1.8, p=.029) (Table 3a, annex).

In UHG the aRR of detectable antibody was statistically significant for the following characteristics: age 30-39 (aRR 3.5, 95% CI 1.6-7.6, p=.002), daily contact with patients with known or suspected COVID-19 infection (aRR 3.1, 95% CI 1.3-6.9, p= 0.009) and male sex (aRR 1.9, 95% CI 1.2-3.0, p= 0.005). (Tables 3b, annex).

Table 2a. Prevalence of SARS-CoV-2 antibodies by participant characteristics, both hospitals

Participant characteristics		Total N	SARS-CoV-2 Ab detected n	% (95% CI)	P-value*
Age groups	18-29	1,350	177	13 (11 – 15)	
	30-39	1,617	168	10 (8.9 - 12)	<0.001
	40-49	1,515	124	8.2 (6.9 – 9.7)	
	50-59	1,001	77	7.7 (6.1 – 9.5)	
	Over 60	304	30	9.9 (6.8 – 14)	
Sex	Female	4,478	422	9.4 (8.6 – 10)	
	Male	1,308	154	12 (10 - 14)	0.013
Ethnicity	Irish	4,444	384	8.6 (7.8 – 9.5)	
	Any other white background	551	62	11 (8.7 – 14.2)	<0.001
	African and any other black background	113	16	14 (8.3 - 22)	
	Asian background	577	107	19 (16 - 22)	
	Other	101	7	6.9 (2.6 - 15)	
Country of birth*	Ireland	4,273	373	8.7 (7.9 – 9.6)	
	United Kingdom	344	32	9.3 (6.5 - 13)	
	India	299	54	18 (14 - 31)	
	Philippines	191	47	25 (19 - 31)	<0.001
	Poland	72	10	14 (6.9 -24)	
	USA	60	3	5.0 (1.0 - 14)	
	Other	548	57	10 (8.0 - 13)	
Education	Primary	29	4	14 (3.9 - 32)	
	Secondary	684	61	8.9 (6.9 - 11)	0.055
	Third level	2,545	283	11 (9.9 - 12)	
	Post-graduate	2,527	228	9.0 (7.9 - 10)	
Role	Admin	803	48	6.0 (4.4 – 7.9)	
	Medical/dental	982	102	10 (8.6 - 13)	
	Nursing/ midwifery	2,064	263	13 (11 -14)	
	Allied health	1,091	73	6.7 (5.3 -8.3)	<0.001
	General support	434	33	7.6 (5.3 - 11)	
	Health care assistant	286	50	18 (13 - 22)	
	Other	127	7	5.5 (2.2 - 11)	
Lives with	Alone	479	28	5.9 (3.9 – 8.3)	
	With others	5,286	546	10 (9.5 - 11)	0.007
	Missing	22	2	9.1 (1.1 - 29)	
Lives with HCW	Yes	1,767	234	13 (12 - 15)	
	No	3,919	332	8.5 (7.6 -9.4)	<0.001
	Missing	101	10	9.9 (4.9 -18)	

* Calculated using the Chi-Square test

Table 2b. Prevalence of SARS-CoV-2 antibodies by COVID-19 related characteristics, both hospitals

Participant characteristics		Total N	SARS-CoV-2 Ab detected n	SARS-CoV-2 Ab detected % (95% CI)	P-value*
Contact of a COVID-19 case	Yes	1,704	325	19 (17 - 21)	<0.001
	No	249	249	6.1 (5.4 -6.9)	
	Missing	10	2	0.2 (0.2-0.5)	
Setting of close contact	Contact at work	1,495	269	18 (16 - 20)	0.002
	Contact outside of work	209	56	27 (21 - 33)	
Workplace exposure	Daily contact with COVID-19 patients	902	136	15 (13 - 18)	<0.001
	Daily contact with patients without COVID	3,245	344	11 (9.6 - 12)	
	No patients contact	1,635	96	5.9 (4.9 – 7.1)	
Previous COVID-19 like symptoms	No symptoms	2,876	92	3.2 (2.6 – 3.9)	<0.001
	Had symptoms	2,911	484	17 (15 - 18)	
Previous COVID-19 PCR test	Yes	2,778	474	17 (16 - 19)	<0.001
	No	3,003	102	3.4 (2.8 – 4.1)	
Previous positive COVID-19 PCR test	Yes	367	350	95.4 (92.7 – 97.3)	<0.001
	No	5,414	226	4.2 (3.7 – 4.7)	

* Calculated using the Chi-Square test

Table 3. Association between risk factors and the presence of SARS-CoV-2 antibodies, both hospitals

Participant characteristics		Unadjusted relative risk (95% CI)	P-value	Adjusted relative risk (95% CI)	P-value
Hospital	Galway	Ref.			
	St. James's	3.7 (3.1 – 4.6)	<0.001	3.7 (3.0 - 4.5)	<0.001
Age groups	18-29	1.7 (1.3 – 2.2)	<0.001	1.4 (1.1 – 1.9)	0.006
	30-39	1.4 (1.1 - 1.8)	0.022	1.2 (0.9 – 1.5)	0.217
	40-49	1.1 (0.8 - 1.4)	0.656	1.0 (0.8 – 1.3)	0.978
	50-59	Ref.			
	Over 60	1.3 (0.9 -1.9)	0.224	1.4 (0.9 – 2.0)	0.112
Sex	Female	Ref.			
	Male	1.3 (1.1 – 1.5)	0.012	1.2 (1.0 – 1.4)	0.046
Ethnicity	Irish	Ref.			
	Any other white background	1.3 (1.0 – 1.7)	0.041	1.3 (1.0 - 1.6)	0.068
	African and other black background	1.6 (1.0 – 2.7)	0.037	1.3 (0.8 – 2.0)	0.299
	Asian background	2.2 (1.8 – 2.6)	<0.001	1.3 (1.0 – 1.6)	0.028
	Other	0.8 (0.4 -1.7)	0.549	0.6 (0.2 – 1.3)	0.177
Country of birth	Ireland	Ref.			Did not enter
	India	2.1 (1.6 – 2.7)	<0.001		
	Philippines	2.8 (2.2 – 3.7)	<0.001		
	United Kingdom	1.1 (0.8 – 1.5)	0.717		
	Poland	1.6 (0.9 – 2.9)	0.119		
	USA	0.6 (0.2 -1.7)	0.324		
	Other	1.2 (0.9 – 1. 6)	0.193		
Education	Primary	1.5 (0.6 – 3.8)	0.365		Did not enter
	Secondary	1.0 (0.8 – 1.3)	0.933		
	Third level	1.2 (1.0 - 1.5)	0.013		
	Post-graduate	Ref.			
Role	Admin	Ref.			
	Doctor\Dental	1.7 (1.3 - 2.4)	0.001	1.2 (0.8 – 1.7)	0.327
	Nursing	2.1 (1.6 – 2.9)	<0.001	1.6 (1.1 – 2.2)	0.007
	HCA	2.9 (2.0 – 4.2)	<0.001	2.0 (1.4 – 3.0)	0.001
	General support	1.3 (0.8 - 2.0)	0.270	0.9 (0.6 – 1.4)	0.687
	Allied HCW	1.1 (0.8 – 1.6)	0.531	0.9 (0.6 – 1.3)	0.635
	Other	0.9 (0.4 – 2.0)	0.837	1.0 (0.5 – 2.1)	0.941
Lives with	Alone	Ref.			

	With others	1.8 (1.2 – 2.6)	0.002	1.5 (1.0 – 2.1)	0.048
Lives with HCW	No	Ref.			
	Yes	1.6 (1.4 -1.8)	<0.001	1.3 (1.1 – 1.5)	0.007
Contact of a COVID-19 case	No	Ref.			Did not enter
	Yes	3.1 (2.7 – 3.6)	<0.001		
Close contact at work **	No	1.5 (1.2 – 1.9)	0.002		Did not enter
	Yes	Ref.			
Workplace exposure to COVID-19 patients	No patients contact	Ref.			
	Daily contact with patients without COVID	1.8 (1.5 – 2.3)	<0.001	1.4 (1.1 – 1.8)	0.008
	Daily contact with COVID-19 patients	2.6 (2.0 – 3.3)	<0.001	1.6 (1.2 – 2.1)	0.002
Previous COVID-19 like symptoms	No	Ref.			Did not enter
	Yes	5.2 (4.2 – 6.5)	<0.001		

**Calculated for close contacts of COVID-19 cases only (n=1,704)

3.4 Discussion

This was a national SARS-CoV-2 seroprevalence study of HCW in two hospitals in Ireland with diverging community and healthcare rates of infection. Our findings showed that differing seroprevalence in the two hospitals reflected the difference in the respective community seroprevalence (24). We found higher SARS-CoV-2 antibody prevalence in SJH in south inner- city Dublin (15%) than in UHG, Galway (4.1%). We identified risk factors for antibody positivity: staff members working closely with patients with known or suspected COVID-19 infection and living with others, and in particular living with other HCW, were a risk factor for seropositivity. Furthermore, HCAs and nurses were at a higher risk.

3.4.1 Overall seroprevalence

Our participants were similar in age and sex to those in other European studies (11) (8) (15). The seroprevalence between SJH and UHG differed by four-fold, reflecting the difference in seroprevalence in the community in the two geo-locations in June 2020 (24). The seroprevalence in SJH was similar to that found in an earlier study in Tallaght University Hospital (26), also in Dublin, suggesting community incidence as the main risk factor for acquisition of COVID-19 infection in HCW. The seroprevalence in both hospitals fell within the wide range previously described in other studies (10) (11) (12) (13) (14) (15), and fell either side of the European estimate of 8.5% found by a meta-analysis published in November 2020 (16). The seroprevalence in the hospital setting was six times the community seroprevalence found in the SCOPI study, performed four months earlier, for both geo-areas of higher and lower community seroprevalence (24). This difference is unlikely to be due to new infections acquired during that time frame given the low community incidence during those months. The increased risk was across all HCW, including those with no direct patient contact. Similar studies have found even bigger differences between hospital and community seroprevalence; the Greek study found HCW seroprevalence between 10-22 times higher than the general population (15). It should be noted that this seroprevalence study was performed during the second wave of the pandemic in Ireland, before the dramatic increase in incidence associated with the third wave of the pandemic.

3.4.2 Seroprevalence by role and type of patient contact

The higher seroprevalence and higher RR amongst HCAs and nurses reflects the degree of proximity to patients that is required by the role- a recognised risk factor for disease acquisition (7) (73). In UHG the seroprevalence was highest amongst doctors. Though this difference was not statistically significant, it may be accounted for, at least in part, by the fact that many doctors move hospital frequently (at least annually, and some after 3-6 months). It is possible that some of the doctors contributing to this higher seroprevalence had acquired infection during work in other hospitals in areas of higher incidence prior to moving to UHG. The seroprevalence amongst nurses in SJH was 21%, which is very similar to the seroprevalence found amongst the nursing staff in the study conducted in Tallaght University Hospital (26). The seroprevalence and aRR were higher in the high-risk group in our study (those with daily contact with patients with known or suspected COVID-19 disease), followed by the

intermediate risk group. Studies have differed on this result; a German study showed a higher seroprevalence among the intermediate risk group with comparison to the high-risk group, potentially due to less scrupulous adherence to infection control precautions including use of personal protective equipment (PPE) on the non-COVID wards (10), and a large Spanish study found no significant correlation between role or direct patient contact and antibody positivity, though community incidence was higher in their setting (11). It is also likely that social factors played a role in these different seroprevalences among HCW sub-groups, including socialisation at work and in the community.

3.4.3 Previous symptoms and testing

In both hospitals, the seroprevalence was higher than the PCR proven diagnoses of COVID-19 infection (15% vs 10.2% in SJH, and 4.1% vs 1.8% in UGH (74)) and higher than the self-reported previous confirmed diagnoses (15% vs 9.6% in SJH and 4.1% versus 2.7% in UHG). In our study 39% of infections were undiagnosed, and 16% of those with positive antibodies reported that they had not experienced symptoms at any stage. Therefore, it is likely that these HCW were working during the infectious period, with potential for onwards transmission to both patients and other staff, as well as to household members. While the proper use of PPE reduces this risk in the hospital setting, the risk of onwards transmission in the household remains high, as well as in interactions in the hospital with both patients and staff where PPE and IPC measures are not fully adhered to. This is especially true for undiagnosed symptomatic infections, which have higher rates of onwards transmission (75). The majority of these HCW reported symptoms at some stage, though we do not know if the reported symptoms coincided with the time of the undiagnosed infection. This highlights the importance of early detection and reinforces the necessity for universal adherence to standard infection control precautions at all times, compliance with transmission-based precautions and appropriate use of PPE including face masks in the hospital setting(76). These findings supported the national recommendation in place at the time to undertake asymptomatic HCW screening on detection of a hospital-acquired case of COVID-19 (77). Considering these findings, regular screening of asymptomatic HCW beyond this setting may also be considered, particularly in areas or times of higher community incidence (78) (79), although the impact or the frequency of serial testing required to have a significant impact on HCW-HCW or HCW-patient transmission, has not been established. (80) (81) (82).

Other studies have also shown that symptomatic individuals were more likely to be antibody positive than asymptomatic individuals (9). In our study there was no significant difference in the proportion of those who were antibody positive when comparing those who had previous positive PCR with symptoms and those who had previous positive PCR without symptoms, although this may be due to small numbers in the asymptomatic group.

Eight-hundred participants reported that they had experienced symptoms at some stage but had never been tested by PCR for COVID-19 infection. Forty-three of these participants (5%) had a detectable antibody, which means that these people possibly continued to work while having symptoms and not seeking a test for COVID-19. Although this proportion of infections is smaller than that of the asymptomatic infections, it calls for enhanced

messaging to all HCW about the importance of self-isolating when presenting with any symptoms consistent with COVID-19 infection and the need to make testing easily accessible to HCW, even when symptoms are mild. This is likely to remain true after completion of vaccination in HCW who are eligible, as current evidence suggests that while vaccination leads to decreased severity of infection (83), the degree to which vaccination will decrease re-infections and onwards transmission is still not clear, hence early case diagnosis will remain important to stop spread in the hospital setting.

3.4.4 Characteristics of and risk factors for antibody positivity

The main risk factors identified to be statistically significantly associated with antibody positivity (in decreasing order of RR) were working in SJH versus UHG, being a HCA or nurse, daily contact with patients (especially those known or suspected to have COVID-19 infection), age 18-29, living with others, in particular living with other HCW, being of Asian background, and being male. Some of these risk factors have also been identified in other studies, including the meta-analysis of European studies (16) (73) (84). In our study, the association between antibody positivity and male sex was only found in the Galway setting. This risk was independent of all other characteristics included in the study, and therefore is likely to be due to social circumstances that were not examined. In our setting, many non-Irish HCW live together, which was accounted for in the adjusted risk. However, it is possible that there are other social factors relating to ethnicity that were not evaluated in our study and that are contributing to this risk seen amongst those of Asian background, which was true in both settings. To the best of our knowledge no other study has identified HCAs in particular as being at risk; on multivariable analysis in our study being a HCA carried the highest adjusted relative risk of antibody positivity, which reflects the work done by a HCA in our setting, which involves constant close patient contact. This role may differ in other settings.

In our study, having daily contact with COVID-19 infected patients had a stronger association with being antibody positive than living with others. These findings are in agreement with the study by Lai Y et al. which found that contact with patients was responsible for 59% of infections in HCW, colleagues with infection accounted for 11%, and community acquired infections accounted for 13% of infections in HCW (7). These findings must be interpreted considering the variation in the intensity of community transmission, and the varying amount of testing being performed in the two communities. We did find, however, that although most reported known close contact events occurred in the work setting, those who had a close contact in the community or household were more likely to be antibody positive than those whose close contact occurred in the hospital setting, assumably because standard and transmission-based precautions including the use of PPE are not generally practiced, or practiced consistently, in the household or community setting, and the potentially ongoing nature of the close contact event in a household. Other seroprevalence studies among HCW have found some correlation between antibody positivity and size of household (11), and having a household contact (85).To the best of our knowledge ours is the first hospital based seroprevalence study to identify living with other HCW as a risk for being SARS-CoV-2 antibody positive. Our findings suggest that a proportion of the HCW

contracting COVID-19 are doing so in their home environment. This was mainly true of the Dublin setting, potentially impacted upon by rental prices and consequential overcrowded accommodation. As mentioned above, the current higher level of community transmission is likely to increase the proportion of infections acquired in the community or household.

While recent studies have highlighted the protective nature of SARS-CoV-2 antibodies against recurrent symptomatic infection for at least 6 months (30) , no recommendations can be made on an individual level for those participants with positive antibodies given the lack of certainty as to when the antibody was acquired, as well as the fact that even asymptomatic recurrence could lead to onwards transmission in the hospital setting. Staff in all hospitals should continue to follow all public health guidelines for infection prevention and control regardless of antibody status.

3.5 Limitations

Our study has several limitations. Firstly, information on COVID-19 symptoms and test results were self-reported and thus could be biased. Secondly, although the uptake rate of 64% overall is good for an opt-in study, there may be a selection bias; it is possible that those who chose not to take part did so due to busier workload, for example, those working on a COVID-19 ward, and therefore with a higher risk of COVID-19 infection. One of the main reasons for overall good recruitment was the incentive of each participant receiving their individual result. This too may introduce a selection bias; those who already know that they have had COVID-19 infection may have been less interested in participating, as well as those who may have already had private antibody testing done elsewhere, which could lead to an under-estimate in the true seroprevalence. Conversely, those who had a previously confirmed infection by PCR may have had more interest in participating to see if they had gained antibodies (and potential immunity). Thirdly, although the communication strategy was an important part of the recruitment process, the study took part during our second wave of the pandemic, and therefore also relied heavily on engagement with IT platforms (email, messenger groups, hospital intranet) and less on face-to-face announcements. The online consent process, questionnaire, and blood test booking system risks exclusion of those who are less literate in information technology (IT). This was identified as a potential limitation from the start, and attempts were made to mitigate this selection bias. Multilanguage information and plain English were used, and groups identified as potentially at risk of exclusion on this basis were targeted directly for inclusion in the study, with small-group sessions to aid consent and questionnaire completion and walk-in clinics for phlebotomy. In the fourth instance, in testing on two different platforms, we chose to prioritise sensitivity over specificity. However, the rate of discordant results was low, and unlikely to have had a significant effect on the results; there were only 21 samples with a positive result on the Abbott Architect assay that did not have a positive result on either the Roche Elecsys assay or the Wantai ELISA. In the fifth instance, this population was surveyed in October, at the start of the second wave of the pandemic in Ireland. A proportion of the HCW workforce in Ireland is transient, and the staff included in the study may not have worked in the same hospital during the first wave of the pandemic, though over 90% of participants in each hospital reported that had been

working in the same hospital during the first wave of the pandemic. And finally, even though the sample size was large, some covariate partners were small thus rendering further analysis lacking in statistical power and precision (e.g. interactions terms or further stratified analysis).

3.6 Conclusion and Recommendations

The overall seroprevalence of antibodies to SARS-CoV-2 was 15% in SJH and 4.1% in UHG, reflecting the difference in community incidence and seroprevalence in each area, and suggesting that the main risk factor for acquisition of COVID-19 infection in HCW is the local community incidence. The HCW seroprevalence was six times the community seroprevalence in each area. Specific risk factors for antibody positivity included being a HCA or nurse, daily contact with patients (especially those known or suspected to have COVID-19 infection), age 18-29 years, living with others, in particular living with other HCW, being of Asian background, and being male. The degree of previously undiagnosed and asymptomatic infections highlights the need for ongoing universal adherence to infection control guidance including the use of appropriate PPE in the hospital setting, as well as the importance of early case detection. It is essential that HCW have easy access to testing, even with mild or no symptoms. As the national COVID-19 vaccination programme is rolled out, we expect that access to testing for HCW will still be critical. Screening of asymptomatic HCW in the setting of hospital-acquired patient infection or outbreaks is important and regular screening of asymptomatic HCW needs to be considered depending on local epidemiology.

This study is a unique comparison between two hospitals in areas of differing community incidence, in which IPC measures were the same. It is the first study, to the best of our knowledge, to specifically delineate the relationship between living with other HCW and risk of antibody positivity. This study is paramount in improving understanding of transmission dynamics and HCW risk factors (demographic, workplace- and household-related) in hospitals in Ireland. The high proportion of undiagnosed infections underscores the importance of all interventions to reduce infection in the hospital setting. This bundle should include robust infection control guidance and adherence to that guidance, with scrupulous attention to standard and transmission-based precautions including the use of appropriate PPE in the hospital setting, easy access to testing for HCW and prompt outbreak investigation. This study will be crucial in informing the vaccination strategy and the roll-out of vaccines to HCW in Ireland. Emerging evidence of reduced transmission of infection by vaccine recipients (86) endorses prompt vaccination of all HCW.

Chapter 4: Comparison of three commercially available assays for the detection of SARS-CoV-2 antibodies in Hospital Healthcare Workers

4.1 Introduction

4.2 Methods

4.2.1 Study Design

4.2.2 Laboratory Assays

4.2.3 Statistical Methods

4.3 Results

4.3.1 SARS-CoV-2 seropositivity in relation to assay type: Abbott and Roche

4.3.2 Abbott SARS-CoV-2 “Grayzone” results

4.3.3 SARS-CoV-2 seropositivity in relation to assay type: Roche and Wantai

4.3.4 SARS-CoV-2 seropositivity over time (previously PCR-positive participants)

4.3.5 Abbott SARS-CoV-2 seropositivity in relation to participant characteristics

4.4 Discussion

4.4.1 Seropositivity in relation to assay type: Abbott and Roche

4.4.2 Does the Abbott grayzone add anything?

4.4.3 Seropositivity in relation to assay type: Roche and Wantai

4.4.4 Seropositivity over time (previously PCR-positive participants)

4.5 Limitations

4.6 Conclusion and Recommendations

Chapter 4: Comparison of three commercially available assays for the detection of SARS-CoV-2 antibodies in Hospital Healthcare Workers**

**see copy of published scientific manuscript in Appendix 2

4.1 Introduction

SARS-CoV-2 antibodies are an excellent indicator of past COVID-19 infection (27) and their presence therefore helps determine the proportion of a population that has been previously infected. As the COVID-19 pandemic progresses, retained sensitivity over time is an important quality in an antibody assay that is to be used for the purpose of population seroprevalence studies. Decline in SARS-CoV-2 antibody response to natural infection over time has been described (33) (34), and different antibodies may wane at different rates. Understanding the duration of antibody response over time is key to understanding the accuracy of epidemiological studies and informing public health pandemic response measures. Regarding longevity of the antibody response there are few published data, and results differ on breadth and duration of response; Chapter 1 (section 1.2) describes in detail the published literature to date comparing the longevity of response of the different commercially available SARS-CoV-2 antibody assays. The aim of this study was to compare the prevalence of anti- SARS-CoV-2 antibodies in the same HCW population in two different assays targeting the anti-nucleocapsid protein; Abbott Architect SARS-CoV-2 immunoglobulin (Ig)G assay and Roche Elecsys Anti-SARS-CoV-2 immunoassay (62) (63) (64). We aimed to also compare antibody prevalence for a subset of samples on an assay targeting the anti-spike protein; the Wantai SARS-CoV-2 Antibody ELISA.

4.2 Methods

4.2.1 Study Design

This was a cross-sectional study of the seroprevalence of circulating antibodies to SARS-CoV-2 in hospital HCW, performed in October 2020 and described in Chapter 3. All staff members of two hospitals were invited to participate in an online self-administered consent process and online questionnaire, followed by blood sampling for SARS-CoV-2 antibody testing. Information collected in the questionnaire included demographic information and information regarding previous COVID-19 symptoms, testing and diagnosis.

4.2.2 Laboratory Assays

All samples were tested by two assays; the Abbott SARS-CoV-2 measuring IgG (referred to here as Abbott) and Roche Elecsys Anti-SARS-CoV-2 measuring total antibodies (referred to here as Roche) (62) (63) (64). The Abbott SARS-CoV-2 IgG assay (Abbott) is a chemiluminescent microparticle immunoassay that detects IgG

antibodies to the nucleocapsid protein of SARS-CoV-2. The Roche Elecsys Anti-SARS-CoV-2 total antibody assay (Roche) is an electrochemiluminescent immunoassay that detects antibodies (including IgG), also to the nucleocapsid protein.

Assay results were interpreted using the manufacturers' recommended assay specific thresholds. Assay threshold ≥ 1.4 (sample to calibrator (S/C) index) for Abbott and ≥ 1.0 (Cut-off index (COI)) for Roche were determined to be reactive and interpreted as antibody positive. The Abbott SARS-CoV-2 IgG grayzone is an additional assay threshold band for potential positivity, suggested by the manufacturer to increase assay sensitivity (Abbott Diagnostics Product Information Letter PI1060-2020) (63), see Table 4 for interpretation of the Abbott S/C index within this study. All samples with an Abbott result of positive or grayzone were tested on a third assay in the National Virus Reference Laboratory (NVRL) using the Wantai SARS-CoV-2 Antibody ELISA (referred to here as Wantai), distributed by Fortress Diagnostics. Wantai is an Enzyme-Linked Immunosorbent Assay (ELISA) for qualitative detection of total antibodies (including IgG and IgM) to the spike protein of SARS-CoV-2.

Table 4 Interpretation of the Abbott S/C index

Interpreted result	**Negative**	**Grayzone**	**Positive**
S/C index	<0.5	0.5 to <1.4	≥1.4

In terms of assay performance, according to the manufacturer's specifications all three assays perform with high sensitivity and high specificity, see Table 5. (87).

Table 5 Summary of assay performance according to manufacturer specifications

	Sensitivity	**Specificity**
Abbott SARS-CoV-2 IgG	100% (95% CI; 95.8 - 100)	99.6% (95% CI 99.0 - 99.9)
Roche Anti-SARS-CoV-2	100% (95% CI; 88.3 - 100)	99.8% (95% CI 99.7 - 99.9)
Wantai SARS-CoV-2 Antibody	96.7% (95% CI 83.3 - 99.4)	97.5% (95% CI 91.3 - 99.3)

4.2.3 Statistical methods

Assay concordance was assessed using Cohen's kappa statistic and McNemar's test for difference in proportions. Cohen's kappa coefficient (κ) measures the level of agreement between assays, taking into account the possibility of the agreement occurring by chance. The κ statistic varies from 0 to 1, where 0 = agreement equivalent to chance, 0.1 – 0.20 = slight agreement, 0.21 – 0.40 = fair agreement, 0.41 – 0.60 = moderate agreement, 0.61 – 0.80 = substantial agreement, 0.81 – 0.99 = near perfect agreement and 1 = perfect agreement. Confidence intervals (CI) for the proportion of participants that were seropositive were computed. Multivariable logistic regression was carried out to assess risk factors for the absence of SARS-CoV-2 IgG antibodies controlling for age, sex, ethnicity/background, type of patient contact, severity of symptoms and number of months since PCR

positive test. Forward stepwise selection was used, the Akaike information criterion (AIC) was used to evaluate model efficiency; HCW role was excluded in the final model. We used R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria) and OpenEpi software version 3.01 (Wilson Score) (88).

4.3 Results

COVID-19 serology test results were available for 5,788 participating HCW (comprising 64% of all staff in two Irish tertiary referral hospitals). The majority of participants were female (77%); median age was 39 years (IQR 30-49). A small proportion (5%) of participants were over 60 years of age. By role, the highest proportion of participants were nursing staff (36%). Characteristics of participants by serology assay are shown in Appendices 4A and 4B.

4.3.1 SARS-CoV-2 seropositivity in relation to assay type: Abbott and Roche

All 5,788 participants were tested using the Abbott SARS-CoV-2 IgG assay and 99.9% (n=5,787) were tested using the Roche Anti-SARS-CoV-2 assay. A considerably lower proportion of participants had a positive antibody on the Abbott assay (4.2%) compared to the proportion that had a positive antibody on the Roche assay (9.5%) (Table 6).

Table 6 Serology result by assay

	Negative	Grayzone	Positive	Total	%	Total	% Positive
Abbott SARS-CoV-2	5325	221	242	5788	4.2%	5567	4.3%
Roche Anti-SARS-	5240	-	547	5787	9.5%	5787	9.5%

Total, total tested; Total valid, total number tested excluding grayzone test result

Assay concordance was moderate (k - 0.53; 95% CI: 0.48 to 0.57) (Table 7). McNemar's test for difference in proportions indicated a systematic difference in the proportion of positive results between the two assays ($p<0.001$). Twenty-four participants tested positive on Abbott but negative on Roche. Of the 24, three also tested positive on the Wantai assay, suggesting possible false negative results on Roche for these three participants. Of these three, one was recently diagnosed with COVID-19 (positive by PCR 16 days prior to serology testing). Of the remaining 21, all tested negative on Wantai; none had had prior PCR-confirmed SARS-CoV-2 infection; 11/21 had never been tested and 10/21 had had a negative COVID-19 PCR test at some stage. Of the 21, nine (43%) reported ever having symptoms of COVID-19 (indicating possible undiagnosed infection), eight of whom had mild symptoms (similar to a cold or less) and one of whom had significant symptoms (similar to influenza but not requiring hospital admission). The interval between date of previous symptoms and date of

serology testing varied; less than one month (n=1), one month (n=1), two months (n=2), three months (n=1), five months (n=1), six months (n=1), seven months (n=2). Analysis was carried out in order to explore the association between participant characteristics and discordant results between the Abbott and Roche assays, there was no significant association observed (data not presented here).

Table 7 Distribution of positive and negative results by serology assay

Roche Anti-SARS-CoV-2	**Abbott SARS-CoV-2 IgG**			**Percentage agreement**	**k statistic (95% CI)**
	Positive	Negative or Grayzone	Total		
Positive	218	329	547	93.9%	K 0.53 (0.48 - 0.57)
Negative	24	5216	5240		
Total	242	5545	5787		

K, Cohen's κappa

4.3.2 Abbott 'Grayzone' results

Four percent of participants (n=221) had results in the Abbott SARS-CoV-2 IgG grayzone. Of those 67% (n=149) tested positive on the Roche assay. Of those with grayzone results, 42% were previously diagnosed with COVID-19 (positive by PCR). There were no participant characteristics that were significantly associated with having grayzone test results.

In order to explore whether the numerical value within the grayzone S/C index range could be used to assist interpretation of the grayzone, arbitrary cut-offs (low, medium, high) were applied, and these were compared to the interpreted results on the Roche assay. There was no correlation observed, as a similar proportion of results within each of the arbitrary cut-offs were positive on the Roche assay (Table 8).

Table 8. Comparison of Abbott SARS-CoV-2 IgG S/C index and Roche Anti-SARS-CoV2 interpreted result, among participants with grayzone results (n=221)

	Abbott SARS-CoV-2	**Roche Anti-SARS-CoV-2**		
	Grayzone	**Negative**	**Positive**	**Positivity**
Arbitrary cut-off	n	**n**	**n**	**%**
Low grayzone (S/C 0.5-0.8)	115	39	76	66.1
Medium grayzone (S/C 0.8-1.1)	62	18	44	71.0
High grayzone (S/C 1.1-1.4)	44	15	29	65.9
Total	221	72	149	67.4

SARS-CoV-2 seropositivity in relation to assay type: Roche Anti-SARS-CoV-2 and Wantai SARS-CoV-2 Ab ELISA

In total, 8.4% (n=485) of participants were tested using both the Roche assay and the Wantai assay.
Assay concordance was almost perfect (k - 0.93; 95% CI: 0.88 to 0.97) (Table 9). There was no evidence of difference in the proportion of positive results between the two assays (p<0.131).

Table 9. Distribution of positive and negative results by assay

Roche Anti-SARS-CoV-2	**Wantai SARS-CoV-2 Ab ELISA**			**Percentage agreement**	**k statistic (95% CI)**
	Positive	Negative	Total		
Positive	386	3	389	97.7%	k- 0.93 (0.88 - 0.97)
Negative	8	88	96		
Total	394	91	485		

K, Cohen's κappa

4.3.3 SARS-CoV-2 seropositivity over time (previously PCR-positive participants)

Three hundred and sixty-seven participants were previously diagnosed COVID-19 positive by PCR. Among those, 41% (150/367) (95% CI 35-46) tested positive on Abbott and 95% (348/367) (95% CI 92-96) tested positive on Roche. There were 93 participants who were previously PCR-positive who had a result in the Abbott grayzone. If the Abbott grayzone was included in positive results, that would increase Abbott positivity to 66% (243/367).

Two-hundred and fifty-nine (71%) previously PCR-positive participants were tested on Wantai, and all 259 had a positive result on the Wantai assay. Results for the Wantai assay are excluded from this section as not all samples were tested on this platform.

The self-reported date of previous positive PCR test was available for 365 (99%) participants. The interval between date of previous positive PCR test and date of serology test ranged from 12 to 231 days (2-33 weeks; 0-7 months). Serology test result by number of weeks since positive PCR test (including the breakdown for those who were symptomatic at the time of PCR testing) is shown in Table 10 and visually represented in Figure 3a, b and c (visual representation excludes participants with grayzone results). We saw a decline in antibody positivity on the Abbott assay from week 21 (day 150) onwards. Figure 4 shows the percentage positivity by time (months) since PCR test, including 95% confidence intervals (CI), showing a decline in antibody positivity on the Abbott assay in month 4.

The majority had a time-interval of six months between positive PCR test and serology testing (61%; n=222). There were 210 participants who had a six-month PCR to serology testing interval, and who were symptomatic at the time of their PCR test; among them positivity was 35% (95% CI 29-41) on Abbott (n=73) and it was 93% (95% CI 89-96) for Roche (n=196).

Seventeen participants (4.6%) with a previously PCR-confirmed infection had negative serology test on both the Abbott and Roche assays, and also tested negative on the Wantai assay. Of the 17, one had recent COVID-19 infection (24 days prior) and the remaining 16 had distant infection (positive PCR was five (n=1) or six (n=15) months prior to serology testing). Characteristics of the 17 participants are shown in Appendix 4C. Among the 17, a lower proportion (88%; n=15) had symptoms at the time of their PCR positive test when compared to the overall PCR-positive subgroup (98%; n=358). A higher proportion were of white Irish background (94% versus 65% in the overall PCR-positive subgroup). Other characteristics did not differ considerably. Analysis was carried out in order to explore the association between participant characteristics and negative serology results; there was no significant association observed (data not presented here).

In order to explore whether the Roche quantitative COI or the Abbott S/C index was close to the positive threshold for these 17 participants, arbitrary cut-offs (low, medium and high negative) were applied to the results

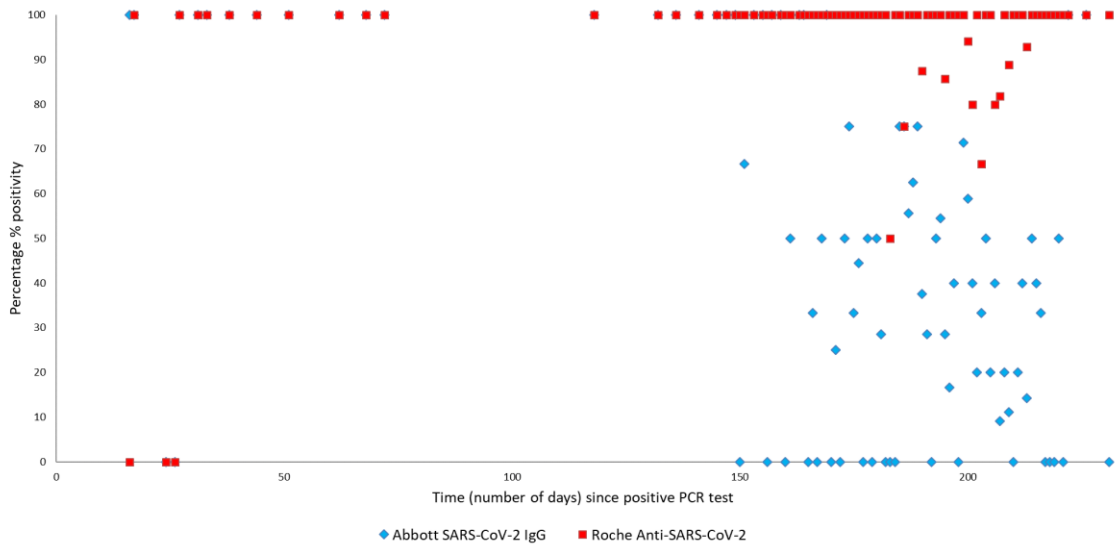
of both assays. For the Roche assay, three participants had results that were close to the positive result threshold (i.e., in the high negative range: COI 0.6-0.9), six had results in the medium negative range (COI 0.3-0.6) and eight had results in the low negative range (COI 0-0.3). For the Abbott assay, all participants had results in either the medium negative range (n=3) or the low negative range (n= 14).

Table 10. Detection of SARS-CoV-2 antibodies by serology assay type with respect to time (number of weeks) since positive PCR test

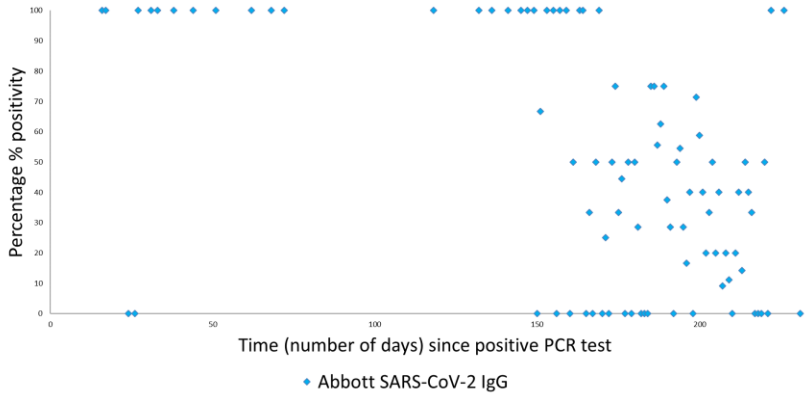
	Previously PCR positive (n=367)										**Previously PCR positive, and had symptoms at time of PCR test (n=340)**									
	Abbott SARS-CoV-2 IgG						*Roche Anti-SARS-CoV-2*				*Abbott SARS-CoV-2 IgG*						*Roche Anti-SARS-CoV-2*			
N weeks	**N**	**G**	**P**	**T**	**% Positive**	**% Positive (valid**	**N**	**P**	**T**	**% Positive**	**N**	**G**	**P**	**T**	**% positive**	**% Positive (valid**	**N**	**P**	**T**	**% Positive**
2	0	0	5	5	100	100	1	4	5	80.0	0	0	2	2	100	100	1	1	2	50.0
3	1	0	0	1	0.0	0.0	1	0	1	0.0	1	0	0	1	0.0	0.0	1	0	1	0.0
4	0	1	2	3	66.7	100	1	2	3	66.7	0	1	2	3	66.7	100	1	2	3	66.7
5	0	0	4	4	100	100	0	4	4	100	0	0	4	4	100	100	0	4	4	100
6	0	0	1	1	100	100	0	1	1	100	0	0	1	1	100	100	0	1	1	100
7	0	0	2	2	100	100	0	2	2	100	0	0	2	2	100	100	0	2	2	100
9	0	0	1	1	100	100	0	1	1	100	0	0	1	1	100	100	0	1	1	100
10	0	0	2	2	100	100	0	2	2	100	0	0	2	2	100	100	0	2	2	100
11	0	0	1	1	100	100	0	1	1	100	0	0	0	0	100	100	0	0	0	100
17	0	0	1	1	100	100	0	1	1	100	0	0	1	1	100	100	0	1	1	100
19	0	0	2	2	100	100	0	2	2	100	0	0	2	2	100	100	0	2	2	100
20	0	0	1	1	100	100	0	1	1	100	0	0	1	1	100	100	0	1	1	100
21	0	1	5	6	83.3	100	0	6	6	100	0	1	5	6	83.3	100	0	6	6	100
22	0	4	7	11	63.6	100	0	11	11	100	0	3	6	9	66.7	100	0	9	9	100
23	3	1	7	11	63.6	70.0	1	10	11	90.9	2	1	7	10	70.0	77.8	0	10	10	100
24	6	5	6	17	35.3	50.0	0	17	17	100	5	5	5	15	33.3	50.0	0	15	15	100
25	9	1	12	33	36.4	57.1	0	33	33	100	9	11	12	32	37.5	57.1	0	32	32	100
26	7	1	7	24	29.2	50.0	1	23	24	95.8	7	9	7	23	30.4	50.0	1	22	23	95.7
27	18	7	23	48	47.9	56.1	2	46	48	95.8	16	7	21	44	47.7	56.8	2	42	44	95.5
28	20	9	18	47	38.3	47.4	2	45	47	95.7	19	8	17	44	38.6	47.2	1	43	44	97.7
29	20	1	24	61	39.3	54.5	6	55	61	90.2	19	16	24	59	40.7	55.8	6	53	59	89.8
30	31	1	9	58	15.5	22.5	4	54	58	93.1	29	18	8	55	14.5	21.6	4	51	55	92.7
31	8	7	6	21	28.6	42.9	0	21	21	100	6	7	6	19	31.6	50.0	0	19	19	100
32	0	1	2	3	66.7	100	0	3	3	100	0	1	2	3	66.7	100	0	3	3	100
33	1	0	0	1	0.0	0.0	0	1	1	100	1	0	0	1	0.0	0.0	0	1	1	100
Total	124	9	150	367	40.9	54.7	19	348	367	94.8	114	88	138	340	40.6	54.8	17	323	340	95.0

Serology test results = N, number negative; G, number grayzone; P, number positive; T, total tested; % positive valid test excluded grayzone results. Table 7 excludes two participants for which time since positive PCR test was unknown.

3A



3B



3C

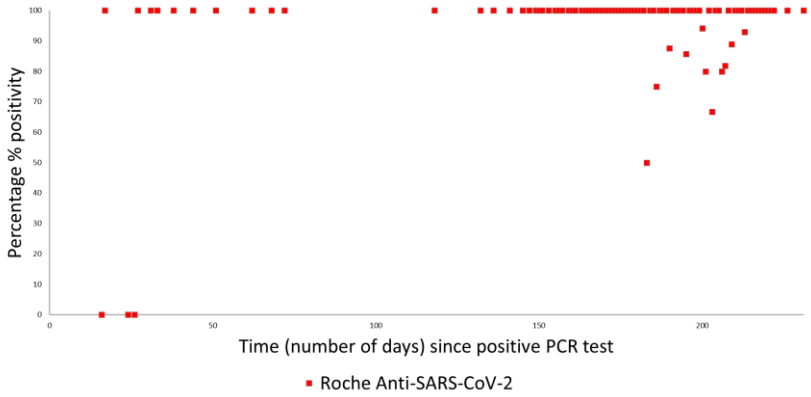
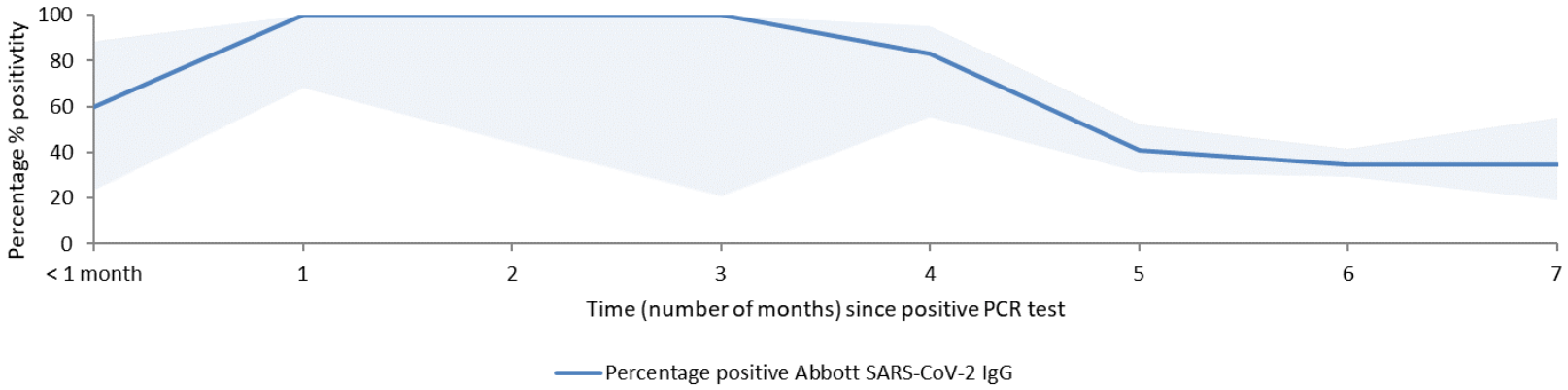


Figure 3 A-C Percentage of participants with detected SARS-CoV-2 antibodies by serology assay with respect to time (number of days) since positive PCR test, among participants who had previous PCR-confirmed COVID-19 infection and had symptoms at the time of their PCR test (n=340). Figure **3A** both assays, **3B** Abbott only, **3C** Roche only.

4A



4B

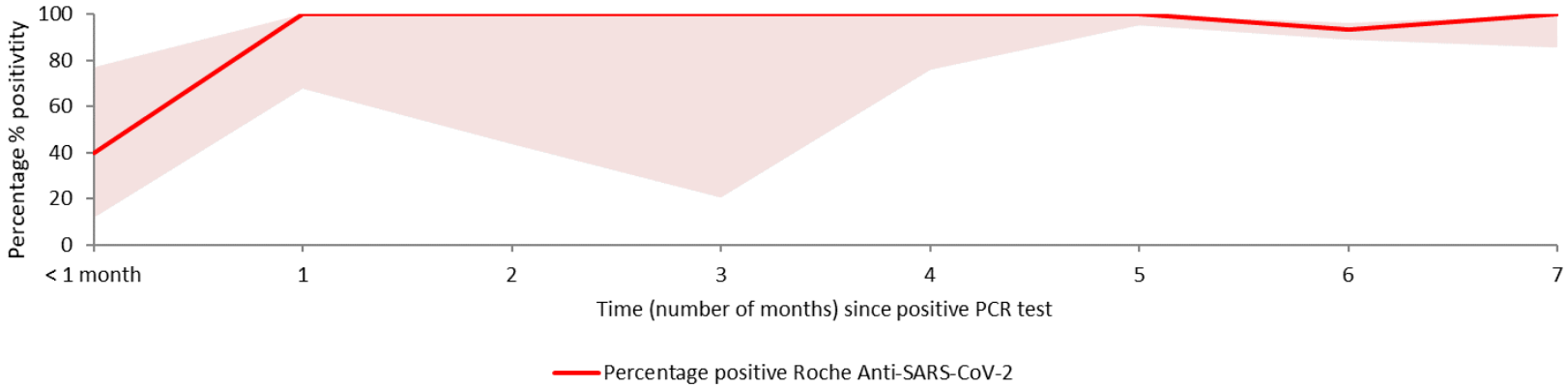


Figure 4A and 4B Percentage % positivity among participants by serology assay with respect to time (number of months) since positive PCR test, among participants who had previous PCR-confirmed SARS-CoV-2 infection and had symptoms at the time of their PCR test (n=340). Shaded area indicates 95% confidence interval (CIs). Note: CIs are wide for months 0-4 and month 7 due to a low number of participants at these testing intervals. Figure **4A** (Abbott only), **4B** (Roche only)

4.3.4 Abbott SARS-CoV-2 seronegativity in relation to participant characteristics

The characteristics of participants with previous PCR-confirmed SARS-CoV-2 infection (n=367) and their serology test results by Abbott SARS-CoV-2 are shown in Appendix 4D and 4E. In total, 45% (n=124) (34% including those who had grayzone results in the total number tested) of participants with previous PCR-confirmed SARS-CoV-2 infection did not have detectable antibodies on the Abbott assay. Univariable and multivariable analysis were carried to out to explore the association between participant characteristics and negative Abbott test result. Table 8 present the results of the analysis. Factors associated with Abbott seronegativity in those with a previous PCR-confirmed infection varied by sex (male adjusted OR (aOR) 0.30; 95% CI 0.15-0.60, *P* <0.001), symptom severity (severe symptoms requiring hospitalisation: aOR 0.19; 95% CI 0.05-0.61, *P* 0.008), ethnicity (Asian ethnicity: aOR 0.28; 95% CI 0.12-0.60, *P* 0.001) and time since PCR diagnosis (increase from five- to six-month interval: aOR 2.06; 95% CI 1.01-4.30, *P* 0.050).

To broaden the analysis, multivariable logistic regression was repeated including participants that had grayzone results. The results of this analysis were similar to the results of the initial analysis and are presented in Appendix 4F. Separate analysis was carried out to explore the association between participant characteristics and Abbott grayzone results; there was no significant association observed (data not presented).

Table 11. Factors associated with Abbott SARS-CoV-2 seronegativity, among participants with previous PCR-confirmed SARS-CoV-2 infection (n=274 participants with valid results)

		All (n)	Negative	Negative %	Unadjusted OR	95% CI	P-value	Adjusted OR	95% CI	P-value
Total		274	124	45.3						
Age group (in years)	18-29	79	41	51.9	*			*		
	30-39	79	43	54.4	1.11	0.59 - 2.07	0.750	0.99	0.46 - 2.15	0.982
	40-49	59	17	28.8	**0.38**	**0.18 - 0.76**	**0.007**	**0.36**	**0.15 - 0.82**	**0.017**
	50-59	45	19	42.2	0.68	0.32 - 1.41	0.301	0.70	0.28 - 1.71	0.429
	60+	12	4	33.3	0.46	0.12 - 1.60	0.238	0.25	0.05 - 1.04	0.065
Sex	Female	201	102	50.7	*			*		
	Male	73	22	30.1	**0.42**	**0.25 - 0.74**	**0.003**	**0.30**	**0.15 - 0.60**	**<0.001**
Ethnicity	Irish	173	94	54.3	*			*		
	Any other white background	35	12	34.3	0.44	0.20 - 0.92	0.033	0.49	0.2 - 1.19	0.118
	Any Asian background	56	14	25.0	**0.28**	**0.14 - 0.54**	**<0.001**	**0.28**	**0.12 - 0.60**	**0.001**
	Any African/black background	7	2	28.6	0.34	0.04 - 1.61	0.200	0.47	0.05 - 3.33	0.449
	Other	3	2	66.7	1.68	0.15 - 36.6	0.067	2.33	0.14 - 70.6	0.566
Close contact with a case of COVID-	Yes	155	65	41.9	0.71	0.44 - 1.15	0.178	0.63	0.34 - 1.16	0.137
	No	117	59	50.4	*			*		
Main type of patient contact^	No patient contact	46	24	52.2	*			*		
	Daily contact non-COVID-19	162	74	45.7	0.77	0.39 - 1.49	0.437	0.79	0.34 - 1.80	0.577
	Daily contact known or	66	26	39.4	0.60	0.27 - 1.27	0.182	0.83	0.32 - 2.14	0.694
Severity of symptoms**	No symptoms	6	3	50.0	0.82	0.15 - 4.61	0.816	0.86	0.12 - 6.29	0.876
	Minor symptoms	113	62	54.9	*			*		
	Significant symptoms	130	55	42.3	**0.60**	**0.36 - 1.00**	**0.051**	**0.50**	**0.27 - 0.92**	**0.028**
	Severe symptoms	23	4	17.4	**0.17**	**0.04 - 0.49**	**0.003**	**0.19**	**0.05 - 0.61**	**0.008**
HCW Role	Administration	18	9	50.0	*					not entered
	Allied health care	34	19	55.9	1.27	0.40 - 4.03	0.686			
	General Support	10	5	50.0	1.00	0.21 - 4.81	1.00			
	Healthcare assistant	24	5	20.8	0.26	0.06 - 0.98	0.053			
	Medical/Dental	62	29	46.8	0.88	0.30 - 2.54	0.809			
	Nursing/Midwifery	124	55	44.4	0.80	0.29 - 2.17	0.653			
	Other	2	2	100.0	NA	NA	NA			
	Less than one month	7	1	14.3	0.25	0.01 - 1.57	0.21	0.11	0.01 - 0.83	0.061

Number of months since PCR positive test**	One month	8	0	0.0	NA	NA	NA	NA	NA	NA
	Two months	4	0	0.0	NA	NA	NA	NA	NA	NA
	Three months	1	0	0.0	NA	NA	NA	NA	NA	NA
	Four months	10	0	0.0	NA	NA	NA	NA	NA	NA
	Five months	57	23	40.4	*			*		
	Six months	168	91	54.2	**1.75**	**0.95 - 3.25**	**0.073**	**2.06**	**1.01 - 4.30**	**0.050**
	Seven months	17	9	52.9	1.66	0.57 - 5.05	0.360	2.67	0.74 - 10.2	0.139

*reference category

P-value calculated using the chi-square test

**Excludes two unknowns

^Participants were asked which type of patient contact describes MOST of their current work (excludes five unknowns)

4.4 Discussion

4.4.1 Seropositivity in relation to assay type: Abbott and Roche

In agreement with recently published data (38), a considerably higher proportion of participants (more than double) had detectable antibodies on the Roche assay compared to the Abbott assay.

In terms of assay performance, according to the manufacturer's instructions both assays perform with high sensitivity and high specificity. Applying the lower bounds of the confidence interval for specificity of the Abbott assay, a maximum false negative rate of 4.2% (n=15) could be expected, therefore 109 out of 124 negative test results among those previously diagnosed with COVID-19 (positive by PCR) cannot be explained by expected test performance; it is possible that SARS-CoV-2 IgG was absent among these individuals. Applying the lower bounds of the of the confidence interval for specificity of the Roche assay, a maximum false negative rate of 0.3% (n=1) could be expected, therefore 18 out of 19 negative test results among those previously diagnosed with COVID-19 (SARS-CoV-2 positive by PCR) cannot be explained by expected test performance. It is possible that some of these individuals did not mount a SARS-CoV-2 antibody response to their infection, however in the published literature the majority of patients have been shown to develop antibodies following natural infection (31) (89) (90), therefore it is likely that the main reason for lack of antibodies in this subset of participants was due to waning of antibody response that was not picked up on testing, particularly on the Abbott assay. Studies have shown that anti-nucleocapsid IgG antibodies may be less likely to develop than IgM antibodies; Zhao et al showed that at day 15 post infection 100.0% had detected total Ab, 94.3% had IgM compared to only 79.8% having IgG Ab (5). Peterson et al and Kaufman et al showed that IgG antibodies were not detected for between 6.3% and 9% of people in the first 2 weeks after PCR confirmation of infection (23) (24) and that this proportion rose to 14% by 99-121 days (92).

Lumley at al demonstrated stable anti-spike IgG antibodies but waning anti-nucleocapsid IgG antibodies over time among HCW in the United Kingdom healthcare service (39), as did Pelleau et al. (93). This may in part explain the Abbott IgG assay's relative performance in detecting past infection, compared to the Roche total antibody assay; the total antibody approach used in the Roche Elecsys system may result in improved and sustained sensitivity suitable for population seroprevalence studies.

Twenty-four participants had detectable antibodies on the Abbott assay but not on the Roche assay, of those three had detectable antibodies on the Wantai assay (one of whom had recent PCR-confirmed infection) and were presumably false negatives on Roche, possibly reflecting differences in test method and target between the Wantai and Roche assays. Among the remaining 21 that did not have detectable antibodies on either the Roche or the Wantai assay there were no participants that had previous PCR-confirmed SARS-CoV-2 infection. While it is possible that these were false positive results, interpretation of positive Abbott results for these 21 individuals is difficult as clinical significance and the duration of persistence of each type of SARS-CoV-2 antibody has yet to be fully elucidated.

4.4.2 Does the Abbott grayzone add anything?

In October 2020, Abbott updated their guidance on their Architect SARS-CoV-2 IgG assay (Abbott Diagnostics Product Information Letter PI1060-2020) to include an optional editable "grayzone" with a S/C index range of 0.5 – 1.39 which they advise "must be interpreted by the clinician in the context of relevant clinical and laboratory information on the patient" (63). The grayzone accounts for a large proportion of the disagreement between the assays. The majority of grayzones were positive by Roche (67%) and Wantai (70%). However, interpretation of this suggested Abbott grayzone result in a clinical setting would not be straightforward and confirmatory testing would still be needed. There was no obvious correlation between increasing or decreasing Abbott grayzone S/C index and the interpreted results from other assays. In an epidemiological study setting such as this, inclusion of all grayzone results as presumptive positives would lead to significant overestimation of seroprevalence. Studies such as ours could be used to estimate the proportion of grayzone results likely to be positive on other testing platforms, however the use of alternative assays with prolonged sensitivity over time would be superior if the primary purpose is to estimate infection ever. It is notable that while studies have shown good protection against reinfection over a six month period following PCR-confirmed COVID-19 infection (30), to the best of our knowledge no studies have yet compared antibody assays in terms of functional immunity.

4.4.3 Seropositivity in relation to assay type – Roche and Wantai

We found almost perfect agreement between the Roche and Wantai assays. A study comparing eight assays found these two assays to provide the highest sensitivities at 98 and 95 percent respectively (94). However, in our study the assay concordance should be interpreted with caution as selection of participants for additional testing on Wantai ELISA assay was heavily biased; only participants who had a positive or grayzone Abbott SARS-CoV-2 IgG result were selected for testing by Wantai assay. The small degree of discordance between these two assays could be due to a number of reasons. Although these two assays both measure SARS-CoV-2 total antibodies, they are based on different methodology (Roche Elecsys Anti-SARS-CoV2 Total AB is based on chemiluminescence and targets the nucleocapsid, whereas the Fortress Wantai Total AB assay is based on ELISA and targets the spike protein). Furthermore, there is a difference in expected performance of these assays in terms of specificity and sensitivity, according to manufacturer guidelines (87).

4.4.4 Seropositivity over time (previously PCR-positive participants)

Of the subset of participants who had previous PCR-confirmed COVID-19 infection, the Roche assay performed better in terms of identifying these participants; 95% tested positive. Only half of these past infections were accurately identified by the Abbott assay. Harley et al. showed a much higher level of agreement between these two assays, though on a smaller number of samples than were tested in our study and at an earlier time point post PCRI-confirmation of infection (95). All previously PCR-positive participants in our study had detectable antibodies on the Wantai ELISA assay, but this subpopulation examination and its inherent selection bias limits strong conclusions from this data.

The majority of participants had a six-month interval between confirmed infection and antibody testing in our study, which correlated with the time period between the peak of the first wave of the pandemic in Ireland and the antibody testing in our study. Although pick-up of these was slightly lower on both assays than the overall pickup of previous infections occurring at any time, the Roche still performed significantly better than Abbott using the 6-month timeframe, identifying 93% versus 46% of infections that occurred six months prior.

The decay in seropositivity on Abbott in our cohort started at 21 weeks (150 days) after confirmed infection, but the small numbers of infection per week prior to this should be noted. This did not change with removal of participants who had no symptoms at the time of infection (Table 7). A study comparing four different serological assays, including the Abbott and Roche assays, showed a decline in the performance of the Abbott assay after 60 days, whereas antibodies were still detected on the Roche assay after 80 days (38). In contrast, another study of the Abbott assay showed a mean of 137 days to loss of positive antibody (39). Kumar et al. showed a much shorter duration of antibody detection using the Roche assay after confirmed SARS-CoV-2 infection in a small cohort of Indian HCW; seropositivity halved by day 30-42 and fell to zero after 50 days (96). In a large US Study, Kaufman et al. showed a decline in IgG antibodies (including on Abbott) to 74% by 2 months after confirmed infection (24). Due to the small numbers of infection per week prior to the 21-week time point in our study, it is possible that this drop-off in Abbott IgG positivity started at an earlier timepoint that was not picked up in our study. While we demonstrate good performance of Roche anti-nucleocapsid assay at extended time points, the comparison with other antigenic targets at time points even more distant from the infection remains obscure.

In a multivariable analysis, the risk of a negative result on the Abbott platform despite previous PCR-confirmed COVID-19 infection increased with interval between infection and antibody testing. Those who had moderate or severe symptoms were more likely to have retained IgG than those who only had mild symptoms. This was consistent with the findings of other researchers who found that IgG was better sustained in persons reporting significant symptoms compared to those who had mild or no symptoms (27) (28) (29). Participants of male sex and Asian ethnicity were also more likely to have sustained Abbott assay detected IgG. Kaufman et al. showed age and male sex to be associated with the probability of persistent IgG serology (24), as did a large Cochrane review conducted in April 2020 (99). Furthermore, Lumley at al. demonstrated that increasing age, Asian ethnicity and prior self-reported symptoms were independently associated with higher maximum anti-nucleocapsid IgG levels (39). This is in keeping with our findings on sex, ethnicity and prior self-reported symptoms, however we did not find any statistically significant correlation with age. The reason for this sustained IgG response in those of male sex is not yet clear but may be related to higher viral load, other indices of severity, or other unknown biological factors not included in this study.

Participants who had a six-month PCR to serology testing interval were twice as likely to be IgG seronegative when compared to participants who had a five-month testing interval. Those who had a seven-month testing were also more likely to be IgG seronegative when compared to those who had a five-month testing interval, but results were not significant. Our findings are consistent with the findings of other

researchers who have demonstrated decline in IgG seropositivity over time, using the Abbott and other IgG antibody assays (33) (26). Further studies may provide better understanding of seropositivity and seroreversion over time, relating to specific assays or type of immunoglobulin measured.

We did not have enough participants with very recent infection to assess the ability of each assay to pick up early infection, however of the eight infections that occurred within four weeks of antibody testing, 8/9 were correctly identified by the Abbott assay and 6/9 were identified by the Roche assay. Higher numbers would be needed to compare these assays specifically in the early stages of infection.

Almost 5% of infections were not identified by either platform. The majority were distant infections (≥6 months ago), and therefore waning immunity may explain the seronegativity. One was a recent infection (PCR positive 24 days prior to serological testing) in a participant who had severe symptoms; this infection may have been too recent to have detectable antibodies on either assay, however four other participants with only minor symptoms and even more recent infections (PCR positive 12-17 days prior to serology), had antibodies detected on both Abbott and Roche assays. Overall, less of these participants with previous confirmed infection and negative serology on both platforms were symptomatic at the time of testing positive by PCR; other studies have shown those without symptoms to be both less likely to develop antibodies, and less likely to have persistent antibodies eight weeks post-infection (28). There were no other participant characteristics that were significantly associated with negative serology, nor were these participants quantitative results (Abbott S/C, Roche COI index) close to the positive result threshold for either assay. Both assays performed below their expected sensitivity based on manufacturers' guidelines, likely due to the time-interval between infection and antibody testing. This should be considered in serological studies, as well as when using antibody testing in the setting of clinical care. As the pandemic progresses, further studies may highlight the sensitivity of different assays with more accuracy in relation to timing of testing.

4.5 Limitations

Our study has several limitations. Firstly, the study was not designed with the primary objective of comparing the serological assays; two anti-nucleocapsid assays +/- additional testing with an anti-spike assay were used to maximise sensitivity in detection of SARS-CoV-2 antibodies. Secondly, information on COVID-19 symptoms and PCR test results were self-reported and thus could be biased. The dates of PCR-confirmed infection (also self-reported) may be inaccurate, furthermore the PCR cycle threshold (Ct) value which would have been a valuable addition to this study was not available. Other variables which would have been valuable to this study but were not available include participant co-morbidities. A small sample size for the 0-4-month PCR to serology test interval prevented meaningful analysis of seropositivity and seronegativity at the early stages post infection.

Our study focused on assays that have SARS CoV-2 nucleocapsid as an antigenic target with only a subpopulation assessed using a spike antibody assay. Given emerging evidence of differences in antibody decay related to the antigenic target a more complete assessment would have been desirable. Such approaches including parallel spike and nucleocapsid assessments may become more relevant in the era of SARS CoV-2 vaccination. In addition, we did not address the neutralising capacity of the antibodies

measured, therefore conclusions about the functional consequences of the presence of such antibodies are limited.

4.6 Conclusion and Recommendations

The Roche assay performed significantly better at picking up those who had ever had a confirmed COVID-19 infection, though both the Roche and Abbott assays were less sensitive than the manufacturers' stated guidelines. Our study findings suggest that, of these two assays directed at anti-nucleocapsid antibodies, Roche is better suited to future population-based serological studies, due to maintained detection of total antibodies up to at least seven months after natural infection with SARS-CoV-2. While maximum sensitivity is achieved using multiple testing platforms, this is not always feasible or cost-effective, and the overall seroprevalence results of our study would have been unchanged if only the Roche assay was used. While we demonstrate good performance of Roche anti-nucleocapsid assay at extended time points, the comparison with other antigenic targets at time points even more distant from the infection remains obscure. The anti-spike assay (Wantai) performed very well on the subset of samples analysed, but further studies are needed to show if it maintains its sensitivity compared to Roche on an unbiased selection of samples.

The risk of a negative antibody result on the Abbott assay despite previous PCR-confirmed SARS-C0V-2 infection increased with increasing interval between infection and time of serological testing; the drop-off was noted 21 weeks/150 days after confirmed SARS-CoV-2 infection. Those of male sex, Asian ethnicity and those with moderate to severe symptoms were more likely to retain IgG on the Abbott assay. Samples with a S/C index within the Abbott grayzone still require additional testing on an alternative assay, and the numerical value within the grayzone S/C index range does not predict the result of confirmatory testing. From our limited numbers of recent infections, it may be possible that the Abbott assay picked up early infection quicker than the Roche assay, but our numbers were too small to determine this. If further studies were to confirm this finding, then the Abbott assay may have a clinical role in early infection where serial PCR fails to diagnose SARS-CoV-2 infection, but clinical suspicion remains high.

Our study adds to the growing literature on serological assays for population-based studies. Further data comparing antibody assays for the detection of antibodies to SARS-CoV-2 are needed to guide the most appropriate use of certain assays in any given situation, especially where use of dual or multiple assays is not feasible or affordable. With the recent introduction of widespread vaccination it is yet to be determined if measuring antibody response to vaccination is meaningful and cost-effective, and if so which assays are superior. Further studies are also needed to delineate the relationship between serological response and functional immunity to SARS-CoV-2 infection, both following vaccination and natural infection, and in immunocompromised patient cohorts. Assays with prolonged sensitivity are likely to be more valuable as the pandemic continues.

Chapter 5: Seroprevalence of antibodies to SARS-CoV-2 in Hospital Healthcare Workers, April 2021

**see copy of published scientific manuscript in Appendix 2

5.1 Introduction

5.1.1 COVID-19 infection in hospital healthcare workers

5.1.2 Antibody response following infection and vaccination

5.1.3 Study sites

5.1.3.1 Study sites - 2021 epidemiology

5.1.4 Aims

5.2 Methods

5.2.1 Study design and participants

5.2.2 Laboratory Methods

5.2.3 Statistical Analysis

5.3 Results 1. Overall SARS-CoV-2 seroprevalence (past infection)

5.3.1. Participation rates and demographics

5.3.2 Previous exposure, symptoms and testing

5.3.3 SARS-CoV-2 seroprevalence by site

5.3.4 SARS-CoV-2 seroprevalence by HCW role

5.3.5 Asymptomatic SARS-CoV-2 infection

5.3.6 Seropositivity by previous diagnosis and symptoms

5.3.7 Undiagnosed SARS-CoV-2 infection

5.3.8 Risk factors for Seropositivity

5.4 Results 2. Antibody response to vaccination

5.4.1 Antibody response to vaccination

5.4.2 SARS-CoV-2 infection post vaccination

5.4.3 Antibody response to infection post vaccination

5.5 Results 3. PRECISE 1 and PRECISE 2 comparison

5.5.1 Comparison of participation rates and participant demographics

5.5.2 Comparison of crude seroprevalence

5.6 Discussion

5.6.1 Participation and demographics

5.6.2 Overall seroprevalence

5.6.3 Seroprevalence by role and type of patient contact

5.6.4 Previous symptoms and testing

5.6.5 Undiagnosed infections

5.6.6 Risk factors for antibody positivity

5.6.7 Antibody response to vaccination

5.6.8 Antibody response (anti-N) to natural infection post vaccination

5.7 Limitations

5.8 Conclusion

5.1 Introduction

5.1.1 COVID-19 infection in hospital healthcare workers

Healthcare workers continue to higher risk of contracting SARS-CoV-2 viral infection than the general population (2) (3) (4). Detectable antibody to SARS-CoV-2 remains the best available indicator of past COVID-19 infection in those who have not been diagnosed with active infection (27). HCW continue to account for a high proportion of the COVID-19 infections notified in Ireland and antibody seroprevalence has been shown in our first study (results Chapter 3) to be up to six times as high as the background community seroprevalence (100) (101) (102). Understanding the transmission and potential immunity dynamics of SARS-CoV-2 in hospitals in Ireland remains important in mitigating transmission at hospital level and adds valuable information to the growing evidence base on the transmission patterns of COVID-19 among HCW both nationally and internationally as the pandemic progresses.

5.1.2 Antibody response following infection and vaccination

Natural infection has been shown to produce humoral and cellular immunity and whilst this may decline over time, durable memory responses are seen; infection-induced immunity has been shown to protect for up to nine months (30) (103). Although the duration of the detectable antibody response to SARS CoV2 can vary depending on the antigenic target and method of detection (35) (38), there is emerging evidence of a rich and sustained memory response in many individuals. Vaccines have been shown to be protective both against infection and against symptomatic disease (47) (104) (105) (106). Vaccination is also associated with lower viral loads and decreased duration of oropharyngeal PCR positivity in subsequent infections, which are very likely to correlate with decreased transmissibility (48). Vaccine-induced immunity produces a more robust response in the adaptive immune system therefore vaccination is likely to produce a sustained immune response with immunological memory and sustained protection, including against variants of concern (VoC) (49) (50). Immunity after natural infection may not protect against re-infection with variants of concern (51), while vaccine-induced immunity is reduced, but not lost, against variants of concern (52) (107) (53). Robust B and T cell responses to vaccination have been shown for both mRNA vaccines and viral vector vaccines (54). Antibody response has been shown to correlate with protective immunity against infection (108).

The spike (S) and nucleocapsid (N) proteins are two of the main immunogens of the coronavirus proteins (109). Commercial SARS-CoV-2 antibody assays can detect antibodies to these structural proteins. Natural infection can produce either anti-N antibodies, anti-S antibodies, both anti-N and anti-S antibodies, or neither antibody. Currently available vaccines against COVID-19 infection produce anti-S antibodies only.

5.1.3 Study sites

The study sites remained the same as for the first cross sectional study (see chapter 1.5). Both hospitals (SJH and UHG) continued to receive patients with COVID-19 infection throughout the second and third waves of

the COVID-19 pandemic in Ireland, and there were no major changes to working structure in either hospital with comparison to the first wave of the pandemic. Both hospitals continued to operate on a ward-based system, with isolation of confirmed cases of COVID-19 on designated COVID-19 wards.

By the time of PRECISE 2, the second cross-sectional study in April 2021, the results of PRECISE 1 were still the most up-to-date and comprehensive data on the SARS-CoV-2 epidemiology at the study sites. In summary; an overall SARS-CoV-2 seroprevalence of 15% in SJH and 4.1% in UHG (HCW seroprevalence six times higher than community seroprevalence (24)); almost 40% of infections previously undiagnosed; 16% of infections asymptomatic; higher risk for seropositivity among healthcare assistants, nurses, those with daily exposure to patients (especially patients with confirmed/suspected COVID-19 infection), those age 18-29 years, those living with other HCW, and those Asian ethnicity and male sex (102) (110).

5.1.3.1 Study sites - 2021 epidemiology

As previously discussed, the community incidence of COVID-19 infection in County Galway was significantly lower than in County Dublin during the first and second waves of the pandemic in Ireland (23). The gap in COVID-19 incidence between Galway and Dublin during the third wave of the COVID-19 pandemic was narrower than during the first two waves; for a 2 week period in late January 2021 the 14-day incidence for Galway approached that of Dublin (59). At the start of this second seroprevalence study in April 2021, the incidence was 181/100,000 in Dublin and 83/100,000 in Galway (61). By the start of April 2021 (third wave of the pandemic in Ireland, (111)) the cumulative incidence of PCR-confirmed infections in HCW in SJH and UHG had risen to 18.5% and 9.2% respectively.

5.1.4 Aims

The purpose of this repeat cross-sectional study (PRECISE 2) was to re-assess the prevalence of anti-SARS-CoV-2 antibodies in HCW in these two hospitals following the third, and larger, wave of the pandemic in Ireland, and to relate risk of COVID-19 infection in HCW to demographic, living arrangements and work-related factors in order to inform ongoing risk reduction activities. We aimed to assess:

1. Changes in overall seroprevalence over a six-month period in these distinct geographical areas.
2. Serological response to COVID-19 vaccination in the vaccinated sub-group.
3. Changes in individual serostatus over time (six-months) for those who participated in PRECISE 1 and PRECISE 2 (see Chapter 6 for results)

5.2 Methods

5.2.1 Study Design and participants

This was a cross-sectional study of the seroprevalence of circulating antibodies to SARS-CoV-2, carried out from the 19th-28th April 2021, with longitudinal linking of participants who also took part in the first serosurvey (PRECISE 1) carried out from the 14th-23rd October 2020. All staff members of both hospitals

(N=9038) were invited to participate in an online self-administered consent process and online questionnaire, followed by blood sampling for SARS-CoV-2 antibody testing in April 2021, in the same manner as PRECISE 1 (102). Information collected in the questionnaire was the same as for PRECISE 1, with the addition of history of COVID-19 vaccination, dates and type of vaccine. Results management was similar, with all participants having their results communicated to them on an opt-out basis (via text message), with the availability of virtual review clinics for any participant who wished to discuss their results in more detail.

All vaccinated study participants received their SARS-CoV-2 vaccine as part of a two-dose regimen, of either the Comirnaty (Pfizer/BioNTech) vaccine, the Vaxzevria (formerly AstraZeneca) vaccine or the Moderna vaccine. The National Immunisation Advisory Committee (NIAC) stated (April 2021) that recipients of the Vaxzevria (AstraZeneca) may not have optimal protection until 15 days after the second dose of vaccine, recipients of the Moderna vaccine may not have optimal protection until 14 days after the second dose of vaccine, and recipients of the Comirnaty (Pfizer/BioNTech) vaccine may not have optional protection until 7 days after the second dose of vaccine (112). It's generally accepted that SARS-CoV-2 vaccine recipients may not have optimal protection until ≥15 days after the second vaccine dose (106). For the purposes of this study, a participant was considered fully vaccinated at ≥14 days after receipt of the second dose of vaccination, in line with Irish guidelines (35). A participant was considered partially vaccinated ≥14 days after receipt of the first dose of vaccination (112,113). A participant was considered to have started a vaccination course once one dose of vaccine had been received at any stage prior to the study.

5.2.2 Laboratory Methods

All samples were tested using the Roche Elecsys anti-SARS-CoV-2 and the Roche Elecsys anti-SARS-CoV-2 S immunoassays detecting total antibodies (including IgG) to the nucleocapsid and spike proteins of the SARS-CoV-2 virus, respectively (64). Thresholds for positive results were as per manufacturers' guidelines (64) (72). Participants with detectable anti-N antibodies were presumed to have had previous natural infection. Participants with detectable anti-S antibodies, and no reported history of COVID-19 vaccination were also presumed to have had natural infection. Participants with detectable anti-S antibodies and a history of COVID-19 vaccination were presumed to have these anti-S antibodies in response to vaccination.

5.2.3 Statistical analysis

Frequencies and percentages were calculated for sociodemographic, epidemiological, and clinical characteristics. Participants were deemed seropositive (i.e., assumed to have had past infection with SARS-CoV-2) if they had detectable anti- N antibodies, or if they had detectable anti-S antibodies but had not been previously vaccinated. Characteristics of those who were seropositive were compared to those who were not seropositive, using the chi-square test. Univariable logistic regression was used to calculate relative risks along with their 95% confidence intervals to assess the association between SARS-CoV-2 seropositivity and characteristics of the study participants. Multivariable regression analysis was conducted to control for negative and positive confounding and to calculate adjusted relative risks (aRR). No explicit finite population correction or reweighting was carried out. All analysis was conducted in Stata 15.1 (StataCorp LCC. 2019. College Station, TX 77845: USA).

5.3 Results 1 SARS-CoV-2 seroprevalence (past infection)

5.3.1 Participation rates and demographics

All staff working in SJH and UHG (9,038 people) were invited to participate in the study. In total 5,108 (57%) blood samples were collected and of those 99% (n=5,085) had a matching questionnaire (questionnaires were deemed to be completed if at least 80% of the questions were answered). In SJH, 63% (2945/4692) of staff participated in both questionnaire and blood sample. In UHG, 49% (2140/4346) of staff participated in both questionnaire and blood sample.

Age and sex of participants were similar in both hospitals (Table 12a). On combined hospital data, 78% of participants were female. Median age was 40 years (IQR 30-49); 5.5% of participants were aged 60 or older. By ethnicity; 75% of participants were white Irish (71% in SJH and 80% in UHG), 12% were Asian (16% in SJH and 6.0% in UHG), and 2.3% were of African or any other black background (2.3% in SJH and 2.2% in UHG). Ninety-one percent of participants lived with other people and 31% lived with other HCW. The highest proportion (37%) of participants were nursing staff, 21% were allied healthcare staff, 14% medical/dental staff (12% in SJH and 17% in UHG), 13% administration staff, 7.2% general support staff (8.3% SJH and 5.7% UHG), 5.7% health care assistants (HCA) and 2.1% other healthcare staff.

Participation by staff grouping was similar in both hospitals; allied health staff had the highest response rate in both hospitals (82% and 67% participation in SJH and UHG respectively) and HCAs had the lowest response rate in both hospitals (42% and 35% participation in SJH and UHG respectively). Participants broadly reflected the HCW breakdown of the staff in both hospitals, with allied health staff minimally over-represented (+5.1%), and HCAs, administration staff, medical/dental staff and nursing/midwifery staff minimally under-represented (for details on participation by HCW role see Tables 5A-D, Appendix).

Table 12a Participant characteristics by hospital and total number of participants, PRECISE 2, April 2021

Participant characteristics		St James's Hospital (N=2,945)		University Hospital (N=2,140)		P-value*	Total (N=5,085)	
		n	**%**	**n**	**%**		**N**	**%**
Age groups	18-29	653	22	455	21	0.431	1,108	22
	30-39	765	26	565	26		1,330	26
	40-49	811	28	603	28		1,414	28
	50-59	565	19	386	18		951	19
	≥60	151	5.1	131	6.1		282	5.5
Sex	Female	2,278	77	1,681	79	0.309	3,959	78
	Male	667	23	459	21		1,126	22
Ethnicity	Irish	2,091	71	1,707	80	<0.001	3,798	75
	Any other white background	257	8.7	219	10		476	9.4
	Asian background	470	16	129	6.0		599	12
	African/other black	69	2.3	48	2.2		117	2.3
	Other	58	2.0	37	1.7		95	1.9
Country of birth	Ireland	2,025	69	1605	75	<0.001	3,630	71
	United Kingdom	134	4.6	161	7.5		295	5.8
	India	225	7.6	68	3.2		293	5.8
	Philippines	198	6.7	16	0.7		214	4.2
	Poland	26	0.9	59	2.8		85	1.7
	USA	21	0.7	34	1.6		55	1.1
	Other	316	11	197	9.2		513	10
Education	Primary	20	0.7	2	0.1	<0.001	22	0.4
	Secondary	409	14	200	9.3		609	12
	Third level	1,280	43	964	45		2,244	44
	Post-graduate	1,236	42	974	46		2,210	43
Role	Admin	403	14	273	13	<0.001	676	13
	Medical/dental	357	12	356	17		713	14
	Nursing/ midwifery	1097	37	794	37		1,891	37
	Allied health	612	21	432	20		1,044	21
	General support	243	8.3	122	5.7		365	7.2
	Health care assistant	179	6.1	112	5.2		291	5.7
	Other	54	1.8	51	2.4		105	2.1
Lives with	Alone	270	9.2	194	9.1	0.603	464	9.1
	With others	2,667	90.	1,943	90.8		4,610	90.7
	Missing	8	0.3	3	0.1		11	0.2
Lives with HCW	Yes	928	32	643	30	0.284	1,571	31
	No	1,964	67	1,448	68		3412	67
	Missing	53	1.8	49	2.3		102	2.0

*Calculated using the chi-square test

5.3.2 Previous exposure, symptoms and testing

COVID-19 related characteristics of participants differed by hospital (Table 1b). Overall, 22% of participating HCW reported that their main type of daily work involved contact with patients with suspected or confirmed COVID-19 (25% of participants in SJH and 19% of participants in UHG), a further 49% reported that their main type of daily work involved contact with patients without suspected COVID-19 infection (46% in SJH and 53% in UHG), and 28% had little or no patient contact (29% in SJH and 28% in

UHG). Symptoms consistent with COVID-19 had occurred at some stage in 47% of SJH staff and 37% of UHG staff. Among the 43% of participants (in both hospitals) who had symptoms at some stage; 30% of these were mild symptoms (equal to a cold or less), 12% were significant symptoms (similar to influenza, bed-ridden), and 0.9% were severe symptoms (requiring hospitalisation). A higher proportion of participants in SJH experienced significant symptoms (14%) when compared to UHG (9.4%).

In terms of self-reported previous laboratory-confirmed COVID-19 infection, 18% of participants in SJH and 14% of participants in UHG reported that they had previously tested positive by PCR. Among those who were previously PCR positive, 21% did not have symptoms at the time of PCR testing (18% in SJH and 26% in UHG).

Table 12b COVID-19 related characteristics by hospital and total number of participants, PRECISE 2, April 2021

Participant characteristics		St James's Hospital **(N=2,945)**		University Hospital **(N=2,140)**		P-value*	Total **(N=5,085)**	
		n	**%**	**n**	**%**	**N**	**%**	
Daily contact with COVID-19 patients	Contact with	726	25	410	19	<0.001	1136	22
	Contact with patients without	1,362	46	1,138	53		2,500	49
	No patient contact	857	29	592	28		1,449	28
Previous COVID-19	No symptoms	1571	53	1342	63	<0.001	2913	57
	Had symptoms	1374	47	797	37		2171	43
	Missing	0	0.0	1	0.0		1	0.0
Severity of symptoms	No symptoms	1571	53	1342	63	<0.001	2913	57
	Mild symptoms	932	32	570	27		1502	30
	Significant	420	14	201	9.4		621	12
	Severe (hospitalised)	21	0.7	26	1.2		47	0.9
	Type of symptoms	1	0.0	0	0.0		1	0.0
	Missing	0	0.0	1	0.0		1	0.0
Previous positive COVID-19 PCR	No	2427	82	1846	86	<0.001	4273	84
	Yes	518	18	294	14		812	16
Symptoms at time of previous	No	95	18	77	26	<0.001	172	21
	Yes	423	82	217	74		640	79
Severity of symptoms at time of PCR test	No symptoms	95	18	77	26	<0.001	172	21
	Mild symptoms	162	31	94	32		256	32
	Significant	248	48	103	35		351	43
	Severe (hospitalised)	12	2.3	20	6.8		32	3.9
	Missing	1	0.2	0	0.0		1	0.1

*Calculated using the chi-square test

5.3.3 SARS-CoV-2 seroprevalence by site

In SJH, seroprevalence among participants was 21%. Seroprevalence was 27% in those who reported having daily contact with COVID-19 patients, 22% in those who reported having daily contact with patients without suspected COVID-19 infection, and 16% in those who had little or no patient contact. In UHG, seroprevalence among participants was 13%. Seroprevalence was 17% in those who reported having daily contact with COVID-19 patients, 15% in those who reported having daily contact with patients without suspected COVID-19 infection, and 6.1% in those who had little or no patient contact.

5.3.4 Seroprevalence by HCW role

By professional subgroup in SJH, seroprevalence was highest among HCAs (39%), followed by nursing/midwifery staff (26%), general support staff (25%), administrative staff (16%), medical/dental staff (15%), allied health professionals (15%), and other healthcare staff (7.4%) (other healthcare staff had small numbers and included those working in education and research, videographers, undefined technicians and others who did not further define their role). Details of seroprevalence (and 95% Confidence Intervals (CI)) by sociodemographic characteristics and by COVID-19 characteristics are shown in table 13a and 13b respectively.

By professional subgroup in UHG, seroprevalence was highest among HCAs (21%), followed by medical/dental staff (17%), general support staff (17%), nursing/midwifery staff (14%), other healthcare staff (12%), administrative staff (7.7%), and allied health professionals (6.7%). Details of seroprevalence and 95% CIs by sociodemographic characteristics and by COVID-19 characteristics are shown in table 13c and 13d respectively.

The combined data for both hospitals showed that HCAs were significantly more likely than other professional groups to be seropositive, with 32% of those participating in the study being seropositive. Seroprevalence in general support staff was second highest at 22%, followed by nursing/midwifery (21%). Prevalence of SARS-CoV-2 seropositivity for both hospitals combined, by participant characteristics and by COVID-19 characteristics are shown in Table 13e and 13f, Annex. The term ‘general support staff’ includes a range of hospital staff roles; by general support role, seroprevalence was highest among catering staff (28%) and domestic/cleaning staff (22%); a detailed breakdown by hospital is shown in Table 13g, Appendix.

Table 13a Prevalence of SARS-CoV-2 seropositivity by participant characteristics, St James's Hospital, PRECISE 2, April 2021

Participant characteristics		Total	SARS-CoV-2 seropositive		
		N	**n**	**% (95% CI)**	**p-value***
Overall		2945	623	21 (20 - 23)	-
Age groups (years)	18-29	653	159	24 (21 - 28)	0.184
	30-39	765	154	20 (17 - 23)	
	40-49	811	157	19 (17 - 22)	
	50-59	565	119	21 (18 - 25)	
	Over 60	151	34	23 (16 - 30)	
Sex	Female	2,278	471	21 (19 - 22)	0.240
	Male	667	152	23 (20 - 26)	
Ethnicity	Irish	2,091	401	19 (18 - 21)	<0.001
	Any other white background	257	56	22 (17 - 27)	
	Asian background	470	122	26 (22 - 30)	
	African or any other black	69	29	42 (30 - 55)	
	Other	58	15	26 (15 - 39)	
Country of birth	Ireland	2,025	386	19 (17 - 21)	0.001
	United Kingdom	134	25	19 (12 - 26)	
	India	225	60	27 (21 - 33)	
	Philippines	198	53	27 (21 - 34)	
	Poland	26	8	31 (14 - 52)	
	USA	21	4	19 (5.4 - 42)	
	Romania	40	12	30 (17 - 47)	
	Nigeria	25	18	72 (51 – 88)	
	Other	251	57	23 (18 - 28)	
Education	Primary	20	7	35 (15 - 59)	<0.001
	Secondary	409	105	26 (22 - 30)	
	Third level	1,280	301	24 (21 - 26)	
	Post-graduate	1,236	210	17 (15 - 19)	
Role	Admin	403	64	16 (12 - 20)	<0.001
	Medical/dental	357	55	15 (12 - 20)	
	Nursing/ midwifery	1097	281	26 (23 - 28)	
	Allied health	612	89	15 (12 - 18)	
	General support	243	60	25 (19 - 31)	
	Health care assistant	179	70	39 (32 - 47)	
	Other	54	4	7.4 (2.1 - 18)	
Lives with	Alone	270	42	16 (11 - 20)	0.019
	With others	2,667	578	22 (20 - 23)	
	Missing	8	3	38 (8.5 - 76)	
Lives with HCW	Yes	928	234	25 (22 - 28)	<.001
	No	1,964	376	19 (17 - 21)	
	Missing	53	13	25 (14 - 38)	

*Calculated using the Chi-square test

Table 13b Prevalence of SARS-CoV-2 seropositivity by COVID-19 related characteristics, St James's Hospital, PRECISE 2, April 2021

COVID-19 related characteristics		Total	SARS-CoV-2 seropositive		
		N	**n**	**% (95% CI)**	**p-value***
Daily contact with COVID-	Contact with COVID-19 patients	726	196	27 (24 - 30)	<0.001
	Contact with patients without COVID-	1,362	293	22 (19 - 24)	
	No patient contact	857	134	16 (13 - 18)	
Previous COVID-19	No symptoms	1571	121	7.7 (6.4 - 9.1)	<0.001
	Had symptoms	1374	502	37 (34 - 39)	
	Missing	0	0	-	
Severity of symptoms	No symptoms	1571	121	7.7 (6.4 - 9.1)	<0.001
	Mild symptoms	932	228	24 (22 - 27)	
	Significant symptoms	420	262	62 (58 - 67)	
	Severe (hospitalised)	21	12	57 (34 - 78)	
	Missing	1	0	-	
Previous positive COVID-19	No	2427	190	7.8 (6.8 - 9.0)	<0.001
	Yes	518	433	84 (80 - 87)	
Symptoms at time of previous	No	95	51	54 (43 - 64)	<0.001
	Yes	423	382	90.3 (87 - 93.0)	
Severity of symptoms at time of PCR test	No symptoms	95	51	54 (43 - 64)	<0.001
	Mild symptoms	162	143	88 (82 - 92.8)	
	Significant symptoms	248	227	91.5 (87 - 94.7)	
	Severe (hospitalised)	12	12	100 (74 - 100)	
	Missing	1	0	-	

*Calculated using the Chi-square test

Table 13c Prevalence of SARS-CoV-2 seropositivity by participant characteristics, University Hospital Galway, PRECISE 2, April 2021

Participant characteristics		Total	SARS-CoV-2 seropositive		
		N	**n**	**% (95% CI)**	**p-value***
Overall		2140	275	13 (11 - 14)	-
Age groups (years)	18-29	455	90	20 (16 - 24)	<0.001
	30-39	565	84	15 (12 - 18)	
	40-49	603	51	8.5 (6.4 - 11)	
	50-59	386	39	10 (7.3 - 14)	
	Over 60	131	11	8.4 (4.3 - 15)	
Sex	Female	1,681	198	12 (10 - 13)	0.005
	Male	459	77	17 (13 - 21)	
Ethnicity	Irish	1,707	194	11 (10 - 13)	0.002
	Any other white background	219	38	17 (13 - 23)	
	Asian background	129	26	20 (14 - 28)	
	African or any other black background	48	10	21 (10 - 35)	
	Other	37	7	19 (8.0 - 35)	
Country of birth	Ireland	1605	181	11 (10 - 13)	<0.001
	United Kingdom	161	19	12 (7.3 - 18)	
	India	68	16	24 (14 - 35)	
	Poland	59	12	20 (11 - 33)	
	USA	34	7	21 (8.7 - 38)	
	Philippines	16	1	6.3 (0.2 - 30)	
	Nigeria	10	1	10 (0.3 - 45)	
	Romania	5	3	60 (14 - 95)	
	Other	182	39	19 (13 - 36)	
Education	Primary	2	0	-	0.485
	Secondary	200	28	14 (10 - 20)	
	Third level	964	133	14 (12 - 16)	
	Post-graduate	974	114	12 (10 - 14)	
Role	Admin	273	21	7.7 (4.8 - 12)	<0.001
	Medical/dental	356	61	17 (13 - 21)	
	Nursing/ midwifery	794	114	14 (12 - 17)	
	Allied health	432	29	6.7 (4.5 - 9.5)	
	General support	122	21	17 (11 - 25)	
	Health care assistant	112	23	21 (13 - 29)	
	Other	51	6	12 (4.4 - 24)	
Lives with	Alone	194	19	10 (6.0 - 15)	0.180
	With others	1,943	256	13 (12 - 15)	
	Missing	3	0	-	
Lives with HCW	Yes	643	106	16 (14 - 20)	0.001
	No	1448	164	11 (10 - 13)	
	Missing	49	5	10 (3.4 - 22)	

*Calculated using the Chi-square test

Table 13d Prevalence of SARS-CoV-2 seropositivity by COVID-19 characteristics, University Hospital Galway, PRECISE 2, April 2021

COVID-19 related characteristics		Total	SARS-CoV-2 seropositive		
		N	**n**	**% (95% CI)**	**p-value***
Daily contact with COVID-19	Contact with COVID-19 patients	410	69	17 (13 - 21)	<0.001
	Contact with patients without COVID-	1,138	170	15 (13 - 17)	
	No patient contact	592	36	6.1 (4.3 - 8.3)	
Previous COVID-19	No symptoms	1342	48	3.6 (2.6 - 4.7)	<0.001
	Had symptoms	797	227	28 (25 - 32)	
	Missing	1	0	-	
Severity of symptoms	No symptoms	1342	48	3.6 (2.6 - 4.7)	<0.001
	Mild symptoms	570	107	19 (16 - 22)	
	Significant symptoms	201	102	51 (48 - 58)	
	Severe (hospitalised)	26	18	69 (44 - 86)	
	Missing	0	0	-	
Previous positive	No	1846	45	2.4 (1.8 - 3.2)	<0.001
	Yes	294	230	78 (73 - 83)	
Symptoms at time of previous	No	77	36	47 (35 - 58)	<0.001
	Yes	217	194	89 (85 - 93.2)	
Severity of symptoms at time of PCR test	No symptoms	77	36	47 (35 - 58)	<0.001
	Mild symptoms	94	83	88 (80 - 94.0)	
	Significant symptoms	103	93	90.3 (83 - 95.2)	
	Severe (hospitalised)	20	18	90.0 (68 - 99.0)	
	Missing	0	0	-	

*Calculated using the Chi-square test

5.3.5 Asymptomatic SARS-CoV-2 infection

The combined data for both hospitals shows that 19% (169/898) of seropositive participants had asymptomatic SARS-CoV-2 infection at some stage (i.e., they were seropositive in this study but reported never having symptoms of COVID-19). Asymptomatic infection by ethnic group was highest among those of African or other black background (36%), followed by white Irish background (20%), other white background (19%), Asian (12%), and other background (9.1%), but confidence intervals overlap. The breakdown was similar by hospital location; breakdown of asymptomatic infection by hospital and HCW role is shown in Table 13h, Appendix.

Among those who had asymptomatic infection (antibody positive and never had symptoms), 33% (55/169) had been previously diagnosed positive by PCR, and 67% (114/169) had not. Among these 114 with previously undiagnosed asymptomatic infection, 73% (83/114) were white Irish, 12% (14/114) were of other white background, 8.8% (10/114) Asian, 4.4% (5/114) African or other black background, and 1.8% (2/114) other background.

5.3.6 Seropositivity by previous diagnosis and symptoms

Sixteen percent (812/5085) of participants reported having had a PCR-confirmed infection with COVID-19 at some stage. Of these, 82% (663/812) were seropositive and 18% (149/812) were seronegative, Table 13f. This meant that 3.6% (149/4187) of all participants who were seronegative had previously had a PCR-confirmed infection with COVID-19. Breakdown by hospital is shown in Tables 13b and 13d. The majority of those reporting a previous confirmed COVID-19 infection were symptomatic at the time of their positive PCR (640/812; 79%), Table 13f. Seroprevalence among those that were symptomatic (576/640; 90%) was significantly higher than seroprevalence among those who were asymptomatic at the time of their confirmed COVID-19 infection (87/172; 51%), ($p<.001$).

In total, 29% (1480/5085) of participants reported that they had experienced symptoms at some stage but had never had a positive PCR test. Of these, 121/1480 (8.2%) were seropositive. Thirty-two of these 121 participants who had never been tested by PCR reported significant COVID-19 like symptoms at some stage.

5.3.7 Undiagnosed SARS-CoV-2 infection

In total, 898 participants (623 in SJH and 275 in UHG) were seropositive. Of these, 235/898 (26%) had never been diagnosed with COVID-19 infection, representing 4.6% (235/5085) of the total study population. The majority of these undiagnosed infections were SJH employees (190/235; 81%). In SJH, 30% (190/623) of those who were seropositive had never previously been diagnosed, and in UHG 16% (45/275) of those who were seropositive had never previously been diagnosed.

Just over half of those with undiagnosed infection (121/235; 51%) had experienced COVID-19 like symptoms at some stage; of those 74% (89/121) had experienced mild symptoms and 26% (32/121) had experienced significant symptoms. This proportion of undiagnosed participants who experienced only mild symptoms (89/121, 74%) was much higher than the proportion of diagnosed participants who experienced only mild symptoms (226/576; 34%) (Table 5E, Appendix).

By ethnicity, the highest proportion (160/235; 68%) of HCW with undiagnosed infection was white Irish, 14% (33/235) were Asian, 12% (28/235) were of other white background, 3% (6/235) were of African or other black background, and 3% (8/235) were of other ethnic background. Most participants with undiagnosed infection reported daily patient contact in their role (192/235; 82%); 36% (84/192) had daily contact with COVID-19 patients and 46% (108/192) had daily contact with patients without suspected COVID-19 infection. By professional role, 42% (98/235) of undiagnosed HCW were nurses, 12% (28/235) were HCAs and 9% (20/235) were in medical/dental roles (of which 18 were doctors). The proportion of undiagnosed participants that were medical/dental professionals was higher in UHG (18%) than in SJH (6.3%). Detailed analysis of undiagnosed infection by HCW role and by hospital location is shown in Table 13i, Appendix.

5.3.8 Risk factors for seropositivity

Characteristics of those participants who were seropositive compared with those who were seronegative for both hospitals combined are shown in Tables 13e and 13f (Appendix). Those of male sex, and those in the 18-29-year age group had a higher seroprevalence; 20% of all participating males had detectable antibody versus 17% of females (p=.008), and 22% of all participants aged 18-29 were seropositive (p<.001). By ethnicity 33% of participants of African or other black background were seropositive, versus 16% of white Irish participants (p<.001). By country of birth, seroprevalence was highest in participants born in Nigeria (54%), Romania (33%), and India (26%), but it should be noted that there were a low number (<100) of participants born in either Nigeria or Romania. Seroprevalence decreased with increasing education level (from 32% for those with primary level education to 15% of those with post-graduate level education, p<.001). Seroprevalence was 32% for HCAs, 22% for general support staff, 21% for nurses, 16% for medical/dental staff, 13% for admin staff, 11% for allied health staff and 10% for other staff (p<.001). Eighteen percent of those living with others were seropositive, compared to 13% of those living alone (p=.008), and 22% of those living with other HCW were seropositive compared with 16% of those not living with HCW (p<.001). Twenty-three percent of those who had daily contact with COVID-19 patients were seropositive, compared to 19% of those who had daily contact with patients without COVID-19, and 12% of those who had little or no patient contact (p<.001).

By hospital, seroprevalence was higher in SJH (21%; 95% CI 20-23) when compared to UHG (13%; 95% CI 11-14) (p<.001). The characteristics of participants who were seropositive in each hospital are shown in Tables 2a-2d. The main differences in seropositivity between the two hospitals were for age, sex, education and living arrangements. For UHG, younger age groups had higher seropositivity (20% seropositivity among 18-29 year-olds versus 10% seropositivity among 50-59 year-olds, p<.001), but this was not observed for SJH (Tables 13a and 13c). For UHG there was also higher seroprevalence amongst those of male sex (17% seropositivity among males versus 12% seropositivity among females, p=.005), but this difference was less pronounced for SJH. For SJH, seroprevalence decreased with increasing education level (p<.001), but this was not observed in UHG. The association between being seropositive and living arrangements was stronger in SJH compared to UHG; in SJH, 22% of participants that were living with other people were seropositive compared to 16% of participants that were living alone (p=.019). The association between seropositivity and living with other HCW was strong in both hospitals. The differences in breakdown by professional subgroup are described above.

On multivariable analysis by hospital, in SJH the aRR of seropositivity was statistically significant for the following characteristics: being a healthcare assistant (aRR 1.9, 95% CI 1.4-2.6, p<.001), being a nurse (aRR 1.5, 95% CI 1.1-2.0, p=0.008), being of African or other black background (aRR 1.8, 95% CI 1.3-2.4, p<.001), secondary level education (aRR 1.5, 95% CI 1.2-1.9, p=0.002), and living with other HCW (aRR 1.2, 95% CI 1.0-1.4, p=0.011), see Table 14a.

In UHG, the aRR of seropositivity was statistically significant for the following characteristics: daily contact with COVID-19 patients (aRR 2.1, 95% CI 1.4-3.3, p=0.001), daily contact with patients without suspected or confirmed COVID-19 (aRR 1.9, 95% CI 1.3-2.9, p=0.001), being aged 18-29 years (aRR 1.7, 95% CI 1.2-2.4, p=0.004), see Table 14b.

On multivariable analysis of the combined hospital data, the adjusted relative risk (aRR) of seropositivity was statistically significant for the following characteristics: working in SJH (aRR 1.5, 95% CI 1.3-1.8, p<.001), being a healthcare assistant (aRR 1.8, 95% CI 1.3-2.3, p<.001), being of African or other black background (aRR 1.7, 95% CI 1.3-2.2, p<.001), secondary level education (aRR 1.4, 95% CI 1.1-1.8, p=0.002), being a nurse (aRR 1.4, 95% CI 1.0-1.8, p=0.022), daily contact with COVID-19 patients (aRR 1.4, 95% CI 1.1-1.7, p=0.002), daily contact with patients without suspected or confirmed COVID-19 (aRR 1.3, 95% CI 1.1-1.5, p=0.013), being 18-29 years of age (aRR 1.3, 95% CI 1.1-1.6, p=0.002), being male (aRR 1.2, 95% CI 1.0-1.4, p=0.016), and living with other HCW (aRR 1.2, 95% CI 1.0-1.4, p=0.007), Table 14c.

Table 14a Association between risk factors and SARS-CoV-2 seropositivity, St James's Hospital, PRECISE 2, April 2021

Participant characteristics		Unadjusted relative risk (95% CI)	P-value	Adjusted relative risk	P-value
Age groups (years)	18-29	1.2 (0.9 - 1.4)	0.174	1.2 (1.0 - 1.5)	0.107
	30-39	1.0 (0.8 - 1.2)	0.677	1.0 (0.8 - 1.3)	0.909
	40-49	0.9 (0.7 - 1.1)	0.437	1.0 (0.8 - 1.2)	0.887
	50-59	Ref.		Ref.	
	Over 60	1.1 (0.8 - 1.5)	0.697	1.0 (0.8 - 1.5)	0.655
Sex	Female	Ref.		Ref.	
	Male	1.1 (0.9 - 1.3)	0.237	1.2 (1.0 - 1.4)	0.104
Ethnicity	Irish	Ref.		Ref.	
	Any other white background	1.1 (0.9 - 1.5)	0.312	1.1 (0.8 - 1.4)	0.516
	Asian background	1.4 (1.1 - 1.6)	0.001	1.1 (0.9 - 1.3)	0.571
	African or other black background	**2.2 (1.6 - 2.9)**	**<0.001**	**1.8 (1.3 - 2.4)**	**<0.001**
	Other	1.3 (0.9 - 2.1)	0.187	1.3 (0.8 - 2.0)	0.249
Country of birth	Ireland	Ref.			not entered
	India	1.4 (1.1 - 1.8)	0.005		
	Philippines	1.4 (1.1 - 1.8)	0.007		
	United Kingdom	1.0 (0.7 - 1.4)	0.908		
	Poland	1.6 (0.9 - 2.9)	0.108		
	USA	1.0 (0.4 - 2.4)	0.999		
	Romania	1.6 (1.0 - 2.5)	0.065		
	Nigeria	3.8 (2.9 - 4.9)	<0.001		
	Other	1.2 (0.9 - 1.5)	0.162		
Education	Primary	2.1 (1.1 - 3.8)	0.020	1.6 (0.9 - 2.9)	0.115
	Secondary	**1.5 (1.2 - 1.9)**	**<0.001**	**1.5 (1.2 - 1.9)**	**0.002**
	Third level	1.4 (1.2 - 1.6)	<0.001	1.2 (1.0 - 1.4)	0.103
	Post-graduate	Ref.		Ref.	
Role	Admin	Ref.		Ref.	
	Doctor\Dental	1.0 (0.7 - 1.4)	0.857	0.9 (0.6 - 1.3)	0.673
	Nursing	**1.6 (1.3 - 2.1)**	**<0.001**	**1.5 (1.1 - 2.0)**	**0.008**
	HCA	**2.5 (1.8 - 3.3)**	**<0.001**	**1.9 (1.4 - 2.6)**	**<0.001**
	General support	1.6 (1.1 - 2.1)	0.006	1.3 (0.9 - 1.8)	0.152

	Allied HCW	0.9 (0.7 - 1.2)	0.559	0.9 (0.7 - 1.3)	0.754
	Other	0.5 (0.2 - 1.2)	0.123	0.4 (0.2 - 1.1)	0.093
Lives with	Alone	Ref.			not entered
	With others	1.4 (1.0 - 1.9)	0.024		
Lives with HCW	No	Ref.		Ref.	
	Yes	**1.3 (1.1 - 1.5)**	**<0.001**	**1.2 (1.0 - 1.4)**	**0.011**
Workplace exposure to COVID-19 patients	No patient contact	Ref.		Ref.	
	Daily contact with patients without COVID-19	1.4 (1.1 - 1.7)	0.001	1.2 (0.9 - 1.4)	0.270
	Daily contact with COVID-19 patients	1.7 (1.4 - 2.1)	<0.001	1.2 (1.0 - 1.5)	0.090
Previous COVID-19 like symptoms	No	Ref.			not entered
	Yes	4.7 (3.9 - 5.7)	<0.001		
Severity of symptoms	No symptoms	Ref.			not entered
	Mild symptoms	3.2 (2.6 - 3.9)	<0.001		
	Significant symptoms	8.1 (6.7 - 9.8)	<0.001		
	Severe symptoms (hospitalisation)	7.4 (4.9 - 11.2)	<0.001		

Table 14b Association between risk factors and SARS-CoV-2 seropositivity, University Hospital Galway, PRECISE 2, April 2021

Participant characteristics		Unadjusted relative risk (95% CI)	P-value	Adjusted relative risk	P-value
Age groups (years)	18-29	**2.0 (1.4 - 2.8)**	**<0.001**	**1.7 (1.2 - 2. 4)**	**0.004**
	30-39	1.5 (1.0 - 2.1)	0.034	1.3 (0.9 - 1.9)	0.120
	40-49	0.8 (0.6 - 1.2)	0.380	0.8 (0.5 - 1.2)	0.304
	50-59	Ref.		Ref.	
	Over 60	0.8 (0.4 - 1.6)	0.570	0.8 (0.4 - 1.5)	0.501
Sex	Female	Ref.		Ref.	
	Male	1.4 (1.1 - 1.8)	0.004	1.3 (1.0 - 1.7)	0.097
Ethnicity	Irish	Ref.		Ref.	
	Any other white background	1.5 (1.1 - 2.1)	0.009	1.3 (1.0 - 1.7)	0.078
	Asian background	1.8 (1.2 - 2.6)	0.002	1.2 (0.8 - 1.8)	0.333
	African or other black background	1.8 (1.0 - 3.2)	0.036	1.3 (0.7 - 2.4)	0.388
	Other	1.7 (0.8 - 3.3)	0.142	1.4 (0.7 - 2.8)	0.328
Country of birth	Ireland	Ref.			not entered
	India	2.1 (1.3 - 3.3)	0.001		
	Philippines	0.6 (0.1 - 3.7)	0.543		
	United Kingdom	1.0 (0.7 - 1.6)	0.841		
	Poland	1.8 (1.1 - 3.0)	0.027		
	USA	1.8 (0.9 - 3.6)	0.080		
	Romania	5.3 (2.6 - 11)	<0.001		
	Nigeria	0.9 (0.1 - 5.7)	0.899		
	Other	1.7 (1.2 - 2.4)	0.001		
Education	Primary	-			not entered
	Secondary	1.2 (0.8 - 1.8)	0.361		
	Third level	1.2 (0.9 - 1.5)	0.168		
	Post-graduate				
Role	Admin	Ref.		Ref.	
	Doctor\Dental	2.2 (1.4 - 3.6)	0.001	0.9 (0.5 - 1.5)	0.660
	Nursing	1.9 (1.2 - 2.9)	0.006	1.0 (0.6 - 1.6)	0. 901
	HCA	2.7 (1.5 - 4.6)	<0.001	1.5 (0.8 - 2.7)	0.180
	General support	2.2 (1.3 - 3.9)	0.005	1.1 (0.6 - 2.1)	0.704

	Allied HCW	**0.9 (0.5 - 1.5)**	**0.622**	**0.6 (0.3 - 1.0)**	**0.053**
	Other	1.5 (0.6 - 3.6)	0.331	0.8 (0.3 - 1.8)	0.533
Lives with	Alone	Ref.			not entered
	With others	1.3 (0.9 - 2.1)	0.188		
Lives with HCW	No	Ref.		Ref.	
	Yes	1.5 (1.2 - 1.8)	0.001	1.1 (0.9 - 1.4)	0.317
Workplace exposure to COVID-19 patients	No patient contact	Ref.		Ref.	
	Daily contact with patients without COVID-19	**2.5 (1.7 - 3.5)**	**<0.001**	**1.9 (1.3 - 2.9)**	**0.001**
	Daily contact with COVID-19 patients	**2.8 (1.9 - 4.1)**	**<0.001**	**2.1 (1.4 - 3.3)**	**0.001**
Previous COVID-19 like symptoms	No	Ref.			not entered
	Yes	8 (5.9 - 10.7)	<0.001		
Severity of symptoms	No symptoms	Ref.			not entered
	Mild symptoms	5.2 (3.8 - 7.3)	<0.001		
	Significant symptoms	14.2 (10.4 - 19.3)	<0.001		
	Severe symptoms (hospitalisation)	19.4 (13.3 - 28.2)	<0.001		

Table 14c Association between risk factors and SARS-CoV-2 seropositivity, both hospitals, PRECISE 2, April 2021

Participant characteristics		Unadjusted relative risk (95% CI)	P-value	Adjusted relative risk (95% CI)	P-value
Hospital	Galway University Hospital	Ref.			
	St James's Hospital	**1.6 (1.4 - 1.9)**	**<0.001**	**1.5 (1.3 - 1.8)**	**<0.001**
Age groups (years)	18-29	**1.4 (1.1 - 1.6)**	**0.001**	**1.3 (1.1 - 1.6)**	**0.002**
	30-39	1.1 (0.9 - 1.3)	0.427	1.1 (0.9 - 1.3)	0.299
	40-49	0.9 (0.7 - 1.1)	0.209	1.0 (0.7 - 1.1)	0.569
	50-59	Ref.		Ref.	
	Over 60	1.0 (0.7 - 1.3)	0.794	1.0 (0.7 - 1.3)	0.948
Sex	Female	Ref.		Ref.	
	Male	**1.2 (1.1 - 1.4)**	**0.007**	**1.2 (1.0 - 1.4)**	**0.016**
Ethnicity	Irish	Ref.		Ref.	
	Any other white background	1.3 (1.0 - 1.5)	0.020	1.2 (1.0 - 1.4)	0.120
	Asian background	1.6 (1.3 - 1.8)	<0.001	1.1 (0.9 - 1.3)	0.206
	African or other black background	**2.1 (1.6 - 2.8)**	**<0.001**	**1.7 (1.3 - 2.2)**	**<0.001**
	Other	1.5 (1.0 - 2.1)	<0.001	1.3 (0.9 - 1.9)	0.145
Country of birth	Ireland	Ref.			not entered
	India	1.7 (1.3 – 2.0)	<0.001		
	Philippines	1.6 (1.3 - 2.1)	<0.001		
	United Kingdom	1.0 (0.7 - 1.3)	0.749		
	Poland	1.5 (1.0 - 2.2)	0.040		
	USA	1.3 (0.8 - 2.2)	0.364		
	Romania	2.1 (1.4 - 3.2)	<0.001		
	Nigeria	3.5 (2.5 - 4.8)	<0.001		
	Other	1.4 (1.1 - 1.7)	0.002		
Education	Primary	2.2 (1.2 – 4.0)	0.014	1.6 (0.9 - 2.9)	0.138
	Secondary	**1.5 (1.2 - 1.8)**	**<0.001**	**1.4 (1.1 - 1.8)**	**0.002**
	Third level	1.3 (1.2 - 1.5)	<0.001	1.1 (1.0 - 1.3)	0.133
	Post-graduate	Ref.		Ref.	
Role	Admin	Ref.		Ref.	
	Doctor\Dental	1.3 (1.0 - 1.7)	0.051	1.0 (0.7 - 1.4)	0.973
	Nursing	1.7 (1.3 - 2.1)	<0.001	1.4 (1.0 - 1.8)	0.022

	HCA	**2.5 (2.0 - 3.3)**	**<0.001**	**1.8 (1.3 - 2.3)**	**<0.001**
	General support	1.8 (1.3 - 2.3)	<0.001	1.2 (0.9 - 1.7)	0.144
	Allied HCW	0.9 (0.7 - 1.2)	0.424	0.8 (0.6 - 1.1)	0.119
	Other	0.8 (0.4 - 1.4)	0.381	0.7 (0.3 - 1.2)	0.134
Lives with	Alone	Ref.			not entered
	With others	1.4 (1.1 - 1.8)	0.010		
Lives with HCW	No	Ref.		Ref.	
	Yes	**1.3 (1.2 - 1.5)**	**<0.001**	**1.2 (1.0 - 1.4)**	**0.007**
Workplace exposure to COVID-19 patients	No patient contact	Ref.		Ref.	
	Daily contact with patients without COVID-19	**1.6 (1.3 - 1.9)**	**<0.001**	**1.3 (1.1 - 1.5)**	**0.013**
	Daily contact with COVID-19 patients	**2.0 (1.7 - 2.4)**	**<0.001**	**1.4 (1.1 - 1.7)**	**0.002**
Previous COVID-19 like symptoms	No	Ref.			not entered
	Yes	5.8 (4.9 - 6.8)	<0.001		
Severity of symptoms	No symptoms	Ref.			not entered
	Mild symptoms	3.8 (3.2 - 4.6)	<0.001		
	Significant symptoms	10.1 (8.6 - 11.9)	<0.001		
	Severe symptoms (hospitalisation)	11.0 (8.5 - 14.3)	<0.001		

5.4 Results 2. Antibody response to vaccination

5.4.1 Antibody response to vaccination

In total, 95% (4854/5085) of participants had received at least one vaccine dose at the time of this study; 14% (724/5085) had received one dose only, 81% (4130/5085) had received two doses, and 4.5% (231/5085) had not received any vaccine doses. In SJH 78% (2290/2945) were fully vaccinated and 19% (546/2945) had received 1st dose of vaccination only, for a total of 96% (2836/2945) participants in SJH having started or completed vaccination. In UHG 86% (1840/2140) were fully vaccinated and 8.3% (178/2140) had received 1st dose vaccination only, for a total of 94% (2018/2140) of participants in UHG having received at least one dose of vaccine. The majority of partially vaccinated HCW (680/724) had received Vaxzevria vaccine; roll out of this vaccine was in February 2021 and therefore these participants were not yet due the second dose due to the longer dosing interval of 12 weeks. Vaccination uptake by ethnicity was similar, with 142/3661 (3.9%, 95% CI 3.3-4.6)) of White Irish participants being unvaccinated, compared to 30/571 (5.2%, 95% CI 3.7-7.4) of Asian participants and 7/110 (6.4%, 95% CI 3.1-13) of Black participants. The vaccines received are shown in Table 15.

Table 15. COVID-19 vaccination status and vaccine brand received by participants, both hospitals, April 2021, PRECISE 2

	Started or completed vaccination^ n/N (%)	Fully vaccinated* n/N (%)
Hospital data combined	**4854/5085 (95.5%)**	**4130/ 5085 (81%)**
	Pfizer 4156/5085 (82%)	Pfizer 4116/5085 (81%)
	Vaxzevria 686/5085 (9.5%)	Vaxzevria 6/5085 (0.1%)
	Moderna 8/5085 (0.2)	Moderna 4/5085 (0.08%)
	I don't know 6/5085 (0.1)	I don't know 2/5085 (0.04%)
SJH	**2836/2945 (96.3%)**	**2290/ 2945 (78%)**
	Pfizer 2305/ 2945 (78%)	Pfizer 2286/ 2945 (78%)
	Vaxzevria 526/2945 (18%)	Vaxzevria 3/2945 (0.1%)
	Moderna 4/2945 (0.1%)	Moderna 1/2945 (0.03%)
UHG	**2018/2140 (94.3%)**	**1840/ 2140 (86%)**
	Pfizer 1849/2140 (86%)	Pfizer 1830/2140 (86%)
	Vaxzevria 161/2140 (7.5%)	Vaxzevria 3/2140 (0.1%)
	Moderna 4/2140 (0.2%)	Moderna 3/2140 (0.1%)
	I don't know 2/2140 (0.09%)	I don't know 2/2140 (0.09%)

^defined as anyone who had received a first dose of vaccine at any stage prior to the study

*defined as ≥14 days after receipt of second dose of vaccine

All fully vaccinated participants (4130/4130, 100%) had detectable anti-S antibodies. Of those that had received one dose of vaccine only, 713/724 (98%) had detectable anti-S antibodies. Of the 724 that had received only one dose of vaccine, 716 of them had received their vaccine >14 days prior to blood sampling for our study, and 713/716 (99.6%) had detectable anti-S antibodies.

5.4.2 SARS-CoV-2 infection post vaccination

In total, 116 participants reported that they had PCR-confirmed SARS-CoV-2 infection since vaccination; of those 82/116 (71%) had received one vaccine dose only and 34/116 (29%) had received their second dose; 23/116 (20%) of these were fully vaccinated i.e. had received their second dose ≥ 15 days before their positive PCR, representing 0.6% (23/4130) of all participants that received two doses and 0.6% (23/4111) of fully vaccinated participants. There were 93 infections in partially vaccinated participants (received only 1 dose of the vaccine or had received their 2nd dose <15 days before their infection) representing 93/724 (13%; 95% CI 11 - 16) of partially vaccinated participants having had a PCR-confirmed infection post vaccination compared to 23/4111 (0.6%; 95% CI 0.4 - 0.8) of fully vaccinated participants (p-value= <.001 (chi-square)). All fully and partially vaccinated participants with breakthrough infection had anti-spike antibodies detected; 21/23 of those fully vaccinated had anti-spike detected at >250u/ml (the other two had antibody levels 133u/ml and 242u/ml respectively) and 88/93 of those partially vaccinated had anti-spike detected at >250u/ml (range of 81-202u/ml for the other 5/93). Characteristics of the participants with breakthrough infection are shown in Table 5F, Appendix. The majority (78%) were working in SJH, 65% were female and by age group the highest proportion (35%) was aged 40-49 years. By ethnicity, just over half (52%) were white Irish and 35% were Asian. Thirty-nine percent had daily contact with COVID-19 patients, and 57% lived with other HCW.

Of the 23 breakthrough infections in fully vaccinated participants, all had received the Pfizer vaccine. (It is noted that this was the most commonly received vaccine as it was the first vaccine to be rolled out, and also that the vaccine schedule for the Pfizer vaccine meant that the other vaccines would have less time for breakthrough cases in fully vaccinated recipients to be observed i.e.. the majority of those that received Vaxzevria vaccine had not yet received their second dose by the time of the study). The median interval between first and second vaccine dose was 21 days (as recommended before 18th January 2021).

5.4.3 Antibody response to infection post vaccination

For the 23 participants with breakthrough infection, the median number of days between second vaccine dose and positive PCR was 30 days (IQR 25-50 days). Five (22%) had symptoms at the time of the positive PCR and 18 (78%) did not have symptoms. While all 23 participants had detectable anti-S antibodies as expected post vaccination, notably, only 6/23 (26%, 95%CI: 11-49) had detectable anti-N antibodies in response to their infection, compared to 663/812 (82%, 95%CI: 79-84, p-value= <.001 (Chi-squared) of all participants in the study with previous PCR-confirmed infection having detectable anti-N antibodies. For the 17 that were anti-N negative after their confirmed infection, the median number of days between PCR positivity and blood sample was 52 days (range 9-67).

5.5 Results 3. PRECISE 1 and PRECISE 2 comparison

5.5.1 Comparison of participation rates and participant demographics

All staff working in SJH and UHG (9,038 people) were invited to participate in both PRECISE 1 (October 2020) and PRECISE 2 (April 2021). Overall, participation was lower in PRECISE 2 (5,085 participants) than in PRECISE 1 (5,787 participants) (102). In SJH, 63% (2945/4692) of staff participated in PRECISE 2; similar to the 65% participation in PRECISE 1, and in UHG, 49% (2140/4346) of staff participated in PRECISE 2, a considerable decrease on 63% participation in PRECISE 1. The decrease in participation rate in UHG was similar across professional subgroups. The distribution of participants in PRECISE 1 and 2 was similar by age group, sex, and education level. By ethnicity, a higher proportion of participants in PRECISE 2 were Asian (12%) when compared to PRECISE 1 (10%). By professional subgroup a higher proportion of participants were allied healthcare workers (19% in PRECISE 1 versus 21% in PRECISE 2; p=0.035), a higher proportion were HCAs (5.7% in PRECISE 2 versus 4.9% in PRECISE 1, p=0.790), and a lower proportion were medical/dental professionals (14% in PRECISE 2 versus 17% in PRECISE 1; p<0.001). A significantly lower proportion of participants in PRECISE 2 had ever experienced symptoms consistent with COVID-19 (47% of participants in SJH, and 37% in UHG) when compared to participants in PRECISE 1 (55% of participants in SJH, and 45% in UHG), (p<.001) (102). In terms of self-reported previous laboratory-confirmed COVID-19 infection, a significantly higher proportion of participants in PRECISE 2 reported having previously tested positive by PCR (18% of participants in SJH, and 14% in UHG), compared to PRECISE 1 (9.6% of participants in SJH, and 2.7% in UHG) (p<.001). (114).

5.5.2 Comparison of crude seroprevalence

A comparison of crude seroprevalence in PRECISE 1 (October 2020) and in PRECISE 2 (April 2021) by participant characteristics and by hospital is shown in Table 16. In SJH, the seroprevalence significantly increased from 15% in PRECISE 1 to 21% in PRECISE 2 (p<.001), and in UHG the seroprevalence significantly increased from 4.1% in PRECISE 1 to 13% in PRECISE 2 (p<.001). For both hospitals combined, seroprevalence increased from 10% in PRECISE 1 to 18% in PRECISE 2. For SJH by ethnic group, the increase in seroprevalence was most pronounced for HCW of African or other black background (from 23% in PRECISE 1 to 42% in PRECISE 2; p=0.020). Increase in seroprevalence in SJH was also significant for those of white Irish ethnicity (from 13% to 19%; p<.001) but the increase was not significant for HCW of Asian ethnicity. By education level in SJH, increase in seroprevalence was highest for HCW with primary education (from 15% in PRECISE 1 to 35% in PRECISE 2) but this increase was not significant due to low numbers of participants in this group. The increase was also high for those of secondary level education, and it was significant (from 13% to 26%; p<.001). By professional subgroup in SJH, increase in seroprevalence was highest for general support staff (from 12% in PRECISE 1 to 25% in PRECISE 2; p<.001) and for HCAs (from 27% in PRECISE 1 to 39% in PRECISE 2; p=0.017). For UHG by ethnic group, the increase in seroprevalence was also most pronounced for HCW of African or other black

background (from 2.1% in PRECISE 1 to 21% in PRECISE 2; p=0.004). Increase in seroprevalence among HCW of Asian ethnicity was significant (from 7.1% to 20%; p<.001) as was the increase among those of White Irish ethnicity (from 3.7% to 11%; p<.001). By professional subgroup in UHG, increase in seroprevalence was also highest for general support staff (from 1.7% in PRECISE 1 to 17% in PRECISE 2; p<.001) and for HCAs (from 6.2% in PRECISE 1 to 21% in PRECISE 2; p=0.001). By age group in UHG, the seroprevalence increased from 4.7% in PRECISE 1 to 20% in PRECISE 2 for younger HCW aged 18-29 years (p<.001), (for SJH a small increase was observed for this age group, but it was not significant).

For both hospitals combined by ethnic group, the increase in seroprevalence was most pronounced for HCW of African or other black background (from 14% in PRECISE 1 to 33% in PRECISE 2; p=0.001). By education level, increase in seroprevalence was highest for HCW with primary education (from 14% in PRECISE 1 to 32% in PRECISE 2) but was not significant due to low numbers in this subgroup (p=0.121). For those of secondary education the increase was also high (from 8.9% to 22%) and it was significant (p<0.001). By professional subgroup, increase in seroprevalence was highest for general support staff (from 7.6% in PRECISE 1 to 22% in PRECISE 2; p<.001) and for HCAs (from 18% in PRECISE 1 to 32% in PRECISE 2; p<.001).

Table 16 Comparison of SARS-CoV-2 seroprevalence October 2020 and April 2021, by hospital

Participant characteristics		St James's Hospital			University Hospital Galway			Both hospitals		
		%	%	%	%	%	%	%	%	%
		Oct-20	Apr-	change	Oct-20	Apr-	change	Oct-20	Apr-21	change
Overall seroprevalence		15	21	6.2	4.1	13	8.8	10	18	7.7
Age groups (years)	18-29	20	24	4.3	4.7	20	15.1	13	22	9.5
	30-39	15	20	5.1	6	15	8.9	10	18	7.9
	40-49	13	19	6.4	3.5	8.5	5.0	8.2	15	6.5
	50-59	13	21	8.1	1.5	10	8.6	7.7	17	8.9
	Over 60	17	23	5.5	2.7	8.4	5.7	9.9	16	6.1
Sex	Female	15	21	5.7	3.5	12	8.3	9.4	17	7.5
	Male	16	23	6.8	6.3	17	10.5	12	20	8.3
Ethnicity	Irish	13	19	6.2	3.7	11	7.7	8.6	16	7.1
	Any other white background	17	22	4.8	6.3	17	11.1	11	20	8.7
	Asian background	24	26	2.0	7.1	20	13.1	19	25	5.7
	African or any other black background	23	42	19	2.1	21	18.7	14	33	19
	Other	13	26	13	-	19	-	6.9	23	16
Country of birth	Ireland	13	19	6.1	3.9	11	7.4	8.7	16	6.9
	United Kingdom	16	19	2.7	3.7	12	8.1	9.3	15	5.6
	India	23	27	3.7	8.2	24	15.3	18	26	7.9
	Philippines	27	27	-0.2	8.0	6.3	-1.8	25	25	0.2
	Poland	29	31	1.8	6.3	20	14	14	24	9.5
	USA	14	19	5.0	-	21	-	5.0	20	15
	Other	16	28	12	4	28	23.5	10	25	15
Education	Primary	15	35	20	0.0	0.0	0.0	14	32	18
	Secondary	13	26	13	3.0	14	11	8.9	22	13
	Third level	18	24	5.5	4.3	14	9.5	11	19	8.3
	Post-graduate	14	17	3.0	4.1	12	7.6	9.0	15	5.7
Role	Admin	10	16	5.9	1.2	7.7	6.5	6.0	13	6.6
	Medical/dental	14	15	1.4	6.9	17	10.2	10	16	6.3
	Nursing/ midwifery	21	26	4.6	4.7	14	9.7	13	21	7.9

	Allied health	10	15	4.5	2.5	6.7	4.2	6.7	11	4.6
	General support	12	25	13	1.7	17	15.5	7.6	22	15
	Health care assistant	27	39	12	6.2	21	14.3	18	32	14
	Other	11	7.4	-3.6	1.4	12	10.4	5.5	9.5	4.0
Lives with	Alone	8.2	16	7.4	3.1	10	6.7	5.9	13	7.2
	With others	16	22	5.7	4.2	13	9.0	10	18	8.1
	Missing	11	38	27	-	-	-	9.1	27	18
Lives with HCW	Yes	21	25	4.2	4.9	16	11.6	13	22	8.6
	No	13	19	6.0	3.8	11	7.5	8.5	16	7.3
	Missing	17	25	8.0	2.1	10	8.1	9.9	18	7.7
Daily contact with COVID-19 patients	Contact with COVID-19 patients	21	27	6.0	7.1	17	9.7	15	23	8.3
	Contact with patients without COVID-	17	22	4.5	4.6	15	10.3	11	19	7.5
	No patient contact	9.5	16	6.1	1.3	6.1	4.8	5.9	12	5.8
Previous COVID-19 symptoms	No symptoms	5.8	7.7	1.9	1.3	3.6	2.3	3.2	5.8	2.6
	Had symptoms	23	37	14	7.5	28	21	17	34	17
Previous positive COVID-19 PCR test	No	6.8	7.8	1.0	1.5	2.4	0.9	4.2	5.5	1.3
	Yes	94.9	84	-11	97.3	78	-19.1	95.4	82	-14

5.6 Discussion

5.6.1 Participation and demographics

Our participants were similar in age and sex to those in other European studies (11) (8) (15). The participation rate was lower for PRECISE 2 compared to PRECISE 1 (56% vs 64%). Overall, however, this is still a good uptake rate for an institutional opt-in study, comparable to other European studies (115) and included representation from all HCW groups, including the traditionally harder-to-reach groups such as general support staff and healthcare assistants, who may not engage as frequently with hospital communications platforms. Importantly, representation was similar across staff roles for PRECISE 1 and PRECISE 2, and overall participant demographics were similar in both studies, and therefore the data are likely to be comparable.

5.6.2 Overall seroprevalence

The overall seroprevalence of antibodies to SARS-CoV-2 in SJH rose from 15% in October 2020 to 21% in April 2021. The overall seroprevalence of antibodies to SARS-CoV-2 in UHG rose from 4.1% in October 2020 to 13% in April 2021. The combined overall seroprevalence for the two hospitals increased from 10% in October 2020 to 18% in April 2021. To the best of our knowledge there are no published studies evaluating SARS-CoV-2 seroprevalence in HCW in Europe in 2021.

In terms of the difference in seroprevalence between the two hospitals, we believe this to be related to local community incidence, to social and demographic factors, and to local work practices. The difference in seroprevalence between the two sites primarily reflects the difference in community incidence, with a corresponding higher seroprevalence seen in the staff SJH, in the more densely populated capital city of Dublin, which has had higher community incidence throughout the pandemic thus far. Other studies have also shown community incidence to be one of the main factors impacting risk to HCW (116) (117). The rise in seroprevalence at both sites reflects the magnitude of the third wave of the pandemic at both sites. The relatively higher increase in seroprevalence in UHG compared to SJH likely reflects the fact that the community incidence in the Galway area approached that of the Dublin incidence during this third COVID-19 wave (59). A higher community incidence means that HCW are more likely to be exposed by the nature of their work which involves direct contact with other people, both patients and other HCW. While this risk disproportionately affected those with closer patient contact, the risk to HCW was higher than in the community, even for those who reported little or no patient contact.

The difference in overall seroprevalence was also likely to have been impacted by differences in hospital infrastructure, work-practices, bed-flow management, and the differing demographic and social factors by HCW role at each site. Broad work-place practices in both hospitals have been similar throughout the pandemic,

including ward-based medical teams and universal use of facemasks. There were no issues with personal protective equipment (PPE) availability in either of the hospitals involved in our study at any stage thus far during the pandemic, and where staff were re-deployed to improve the hospital's capacity to deal with the outbreak, staff were not deployed to areas that would have been outside of their scope of practice, and all staff had training on the correct use of PPE. Both hospitals experienced multiple outbreaks during the 3rd wave of the pandemic, however the absence of real time genomic sequencing precludes identifying the role of hospital outbreaks in influencing the overall seroprevalence. It is also difficult to identify the contribution of the workplace versus the community/ household/ social factors to the attributable risk to HCW. It is likely that many of these transmissions took place in the household, where higher attack rates are seen (118). It is also unclear as to the timing of arrival of different VOCs to each location, and the potential impact of this on changes in overall seroprevalence.

The overall seroprevalence was higher than the European average of 8.5% in a recently published meta-analysis (16), however this meta-analysis only took into account studies published up until August 2020. Individual studies across Europe in the first year of the pandemic showed a wide range of SARS-CoV-2 seroprevalence among HCW (1-45%) (11) (15) (10) (12) (13) (14). A recently published large study in Italian HCW showed a seroprevalence of 12%, however the serological testing was conducted in April and May 2020 (115). Assumably the seroprevalence amongst HCW has risen across the rest of Europe since then, as our study shows it has in Ireland, however there are no published data for comparison.

Our study compared seroprevalence among the same HCW group at two points in time six months apart. A German study evaluated seroprevalence at 3 points in time, all in 2020, and found low seroprevalence among HCW at all three points in time (119). A Japanese study found much lower seroprevalence rates both before and after their second wave of the pandemic (<1% at both points in time, also both in 2020) (120).

5.6.3 Seroprevalence by role and type of patient contact

Daily contact with patients with known/suspected COVID-19 infection was associated with higher seroprevalence, followed by daily contact with patients without known or suspected COVID-19 infection. Having little or no patient contact carried the lowest seroprevalence. This repeated the findings of PRECISE 1 and has also been shown by other studies (121) (122) (123). In terms of working role, being a nurse or a HCA carried a higher aRR, likely also reflecting the close patient contact involved in performing these roles. The highest seroprevalence was seen amongst HCAs; this was also a key finding of PRECISE 1, and this seroprevalence almost doubled over the six months between PRECISE 1 and 2. This finding has also been shown in other large European studies (115).

The seroprevalence among general support staff (which includes domestic and catering staff, maintenance, security and porters) trebled from PRECISE 1 to PRECISE 2, giving them the second highest seroprevalence of

any working role in PRECISE 2. This increase was in both locations and was across all groups in this category (Table 2f, Annex). This was possibly related to outbreaks amongst these staff groups, though it was not clear whether these outbreaks were related to the workplace or not. There may be improper compliance with use of PPE, and fatigue with ICP as the pandemic has progressed. There are likely to also be other social factors involved that our study was not designed to assess.

The seroprevalence amongst medical staff showed one of the largest increases in UHG (from 6.9% to 17%) (Table 5). This may be related to social practices; many doctors working in UHG are not from Galway, and live and socialise together (in UHG 53% of doctors reported living with other HCW compared to 43% in SJH). In SJH, the smallest relative increase between PRECISE 1 and PRECISE 2 (from 14% to 15%) was amongst the medical staff. This resulted in medical staff having the joint-lowest seroprevalence of any role group in SJH by April 2021. Although the overall uptake among medical staff was similar to PRECISE 1, including of those who were seropositive in PRECISE 1, it is possible that medical staff who knew they had already had COVID-19 infection were less interested in participating in the study and availing of serology testing. Other studies have shown that medical staff are more likely to get vaccinated promptly against other infectious diseases (124), including influenza; occupational health data from SJH in 2019 showed that 60% of doctors received the influenza vaccine compared to 23% of nursing staff. In our study, 93% of doctors had completed vaccination and 99% (684/692) had started vaccination, compared to only 81% of all staff having completed vaccination by the time of the study. This may indicate that doctors sought vaccination quicker than other role groups after its roll-out but does not explain the difference between the two hospitals. Overall this data shows that medical staff in SJH were less likely to be infected, and more likely to be diagnosed when they did get infected.

5.6.4 Previous symptoms and testing

Only 82% of participants with previous PCR-confirmed infection had detectable antibodies, compared to 95% of PRECISE 1 participants. This may be related to antibody waning over time for those in whom the reported infection occurred in the first wave of the pandemic. There may also be participants who were vaccinated, and therefore the anti-S antibody detected in PRECISE 2, assumed to be due to vaccination, may have been present before vaccination in response to previous infection. The longitudinal analysis of those participants common to PRECISE 1 and 2 contains more detail on loss of antibody in those who were seropositive in PRECISE 1.

Nineteen percent of participants with detectable antibodies reported never having experienced symptoms that were consistent with infection with COVID-19, compared to 16% in PRECISE 1. This falls within the broad range reported by other studies (125). Those who had a symptomatic infection had a higher rate of antibody positivity than those who had an asymptomatic infection (90% versus 51%). This is also in keeping with other published data (126).

Over a quarter of participants reported having COVID-19 like symptoms at some stage but never having a positive PCR. This highlights the potential overlap in symptoms with other circulating viruses, including rhinoviruses which were circulating widely over the winter of 2020/21 in Ireland, and is a reminder of the impossibility of clinically excluding COVID-19 infection in HCW with symptoms, including in those with only mild symptoms, especially over the winter period. It also highlights the complexity involved in developing case definitions and testing guidelines.

5.6.5 Undiagnosed infections

In both hospitals, the seroprevalence was higher than the known PCR-confirmed diagnoses of COVID-19 infection of the same timeframe (21% vs 18% in SJH, and 13% vs 9.2% in UHG) though this gap was narrower than for PRECISE 1, assumably due to increased awareness and testing. Twenty-six percent of the infections in our study were undiagnosed, with 4.6% of all participants having had an undiagnosed infection. Almost half of these undiagnosed infections were asymptomatic (which was significantly higher than the rate of asymptomatic infections in those who had diagnosed COVID-19 infection). This proportion of undiagnosed infection has decreased from 39% in PRECISE 1, however still a quarter of infections had been undiagnosed despite both hospitals having onsite PCR testing available to HCW with symptoms or close contact with a confirmed case of COVID-19 from mid-March 2020. Although the majority of these infections were associated with only mild symptoms, it is still possible that these undiagnosed HCW were working during the infectious period, with potential for onwards transmission to patients and other staff members if proper use of PPE and other infection prevention and control (IPC) measures were not strictly adhered to. Easy access to testing, early detection of infection, and ongoing adherence to standard infection control precautions at all times, as well as the appropriate use of PPE including face masks in the hospital setting, irrespective of symptoms remain important (76). This finding also supports the recommendation for screening of asymptomatic staff, including vaccinated staff when a patient case of hospital-acquired infection, or hospital outbreak of infection with COVID-19 occurs. The exact role and methodology of routine asymptomatic screening, either widespread or in certain HCW, remains to be defined.

5.6.6 Risk factors for antibody positivity

The main risk factors identified to be statistically significantly associated with antibody positivity (in decreasing order of aRR) were being a HCA, being of Black ethnicity, working in SJH, having secondary level education as opposed to post-graduate level education, being a nurse, having daily contact with patients (especially those known or suspected to have COVID-19 infection), being age 18-29, living with other HCW and being male. When broken down by hospital, the main risk factors identified to be significantly associated with SARS-CoV-2 antibody positivity differed. Seroprevalence by age and sex were similar to previously published literature, and similar to the findings of PRECISE 1 (102) (110) (16). Similar findings of increased risk direct patient contact,

the role of HCA, and working with patients with COVID-19 infection have also been reported in the literature (15) (16) (73) (7).

Apart from the changes in seroprevalence by role discussed above, there were two main new findings on multivariate analysis from PRECISE 2 in comparison to PRECISE 1. Firstly, level of education emerged as an independent risk factor for seropositivity, with lower level of education being associated with higher seropositivity. Lower socio-economic status has been previously noted to correlate with increased risk of COVID-19 infection, and increased risk of poor clinical outcomes (127) (128). It is also associated with HCW role.

The second notable new finding was a change in the seropositivity by ethnic group; the seroprevalence amongst those of Black ethnicity trebled (from 14% (16/113) to 43% (39/117), for an aRR of 1.7 (95% CI 1.3-2.2, p<.001). They were also more likely to be asymptomatic, but not more likely to have an undiagnosed infection. The seroprevalence amongst those of Romanian nationality was also high for both hospitals combined, though numbers were small. Those of Asian ethnicity had a higher risk of seropositivity in PRECISE 1, but this finding was no longer significant in PRECISE 2. Both of these ethnic groups, as well as other minority ethnic groups which were likely under-represented in our study, have been shown to have increased risk in other studies (16) (129) (84) (130). There are likely to be social factors contributing to these ethnicity-related findings in both hospitals that our study did not measure.

Living with other HCW carried an increased risk for seropositivity, similar to our previous findings. This supports the theory that a proportion of the HCW contracting COVID-19 are doing so in their home environment. This finding was stronger in SJH than in UHG, where the community incidence was higher and the density of shared living space is also likely to be higher due to smaller spaces and more expensive accommodation in the capital city. Other studies have found correlation between size of household and antibody positivity (11) as well as higher risk of COVID-19 with a known household contact (85). To the best of our knowledge ours is the first study to comment on a significant risk of antibody positivity in HCW living with other HCW. This finding was common to both PRECISE 1 and 2.

5.6.7 Antibody response to vaccination

Most participants were either fully or partially vaccinated. All fully vaccinated participants, and the majority of partially vaccinated participants, had detectable anti-S antibodies. Other studies have also shown high rates of seropositivity after both first and second dose vaccination (113) (131) (132) .

There were less infections in those fully vaccinated than in those partially vaccinated (13% versus 0.6% of participants reported infection post first and second vaccine respectively), despite the fact that almost all of these participants had detectable antibodies. The 23 breakthrough infections, in 0.6% of the fully vaccinated study population, are in keeping with the rate of breakthrough infections experienced internationally (133). These

breakthrough infections serve as a reminder that vaccination does not prevent infection acquisition, even in the setting of confirmed serological response to vaccination. One study showed that a quarter of vaccinated individuals living with an infected individual acquired COVID-19 infection (134). Most of those with breakthrough infections had no symptoms, in keeping with the literature on vaccine effectiveness in reduction of severe symptoms and hospitalisation (50) (135) however the numbers are too small for any statistical comparison with symptoms in those who were unvaccinated. While some studies show that vaccinated individuals are less likely to onwards transmit COVID-19 once infected (136), this reduction in transmission depends on the variant (137) it would be prudent for all IPC measures to remain in place in the hospital setting, including for vaccinated HCW, while research is ongoing into the effects of vaccination on infection acquisition and onwards transmission, including with VOCs. Vaccinated HCW should not be exempt from measures discussed above in relation to minimising the rate of undiagnosed infections (access to testing, adherence to standard IPC precautions and inclusion in screening of asymptomatic staff in the case of a hospital outbreak).

5.6.8 Antibody response (anti-N) to natural infection post vaccination

It is also notable that of the 23 confirmed infections in fully vaccinated participants, only 6 of these participants had developed anti-N antibodies in response to their infection. Of the 17 that were anti-N negative, median number of days between PCR positivity and sampling mid-point was 52 (range 9-67) so it is surprising that the majority of these had not mounted an anti-N antibody response (89). This low number of seroconversions might suggest that anti-N antibodies may be insensitive as a marker of natural infection post vaccination. It raises the questions of whether the pre-existing spike antibodies alter the way the virus interacts with the immune system, with less production of anti-N antibodies. The numbers are very small but further research is warranted as this would be a very important point as we try to measure seroconversion and seroprevalence going forward in a post-vaccination era. Studies continue to rely on anti-N as a marker of seropositivity related to natural infection, including in vaccinated individuals (138). To the best of our knowledge there are no published data to date that have identified a comparative reduction in anti-N seroconversion following natural infection in vaccinated individuals. Further research of individuals with well-defined vaccine breakthrough infections are required, as this information will be critical in determining how best to assess seroprevalence in vaccinated cohorts. (See letter to the Editor of Journal of Infection in Appendix 2.1; Publications based on this research, https://doi.org/10.1016/j.jinf.2021.08.012).

5.7 Limitations

This study has many of the same limitations as the cross-sectional study described in Chapter 3, as well as limitations particular to this component of the study. Again, information on COVID-19 symptoms and PCR test results were self-reported and thus could be biased. Secondly, although the uptake rate was good for an opt-in study, it was lower than the uptake for PRECISE 1; declining interest in research in the area of COVID-19 is a

natural phenomenon as the pandemic progresses. Many staff at this point in time already knew they had been infected and therefore may have less interest in participating and availing of serology testing. Most staff had been vaccinated and therefore may also have a degree of comfort that produces less interest in knowing their antibody status. PRECISE 2 took place during the third wave of the pandemic in Ireland, which was the largest in magnitude and the longest. Other limitations include that WGS testing results were not available, particularly for those infected after full vaccination, and also that information on biological factors, e.g. co-morbidities, was not available. Although the communication strategy was an important part of the recruitment process, the study took part during our third wave of the pandemic, during Level 5 restrictions - the highest level of COVID-19 national restrictions - and therefore also relied heavily on engagement with information technology (IT) platforms (email, messenger groups, hospital intranet) and less on face-to-face announcements. Thirdly, as with all opt-in studies, there may be a selection bias. Those who had been vaccinated may have had less interest in participating due to less curiosity about their own serostatus, and therefore we may have underestimated the overall vaccination status of the workforce. Conversely, those who were unvaccinated may have had a fear of having to announce their vaccine-status to the study team, despite results not being linked to occupational health records, and those who had been vaccinated may have been more likely to participate as they may be more likely to have health seeking behaviour. The online consent process, questionnaire, and blood test booking system risks exclusion of those who are less literate in IT as discussed in Chapter 3. We do not have individual level information on reasons for non-participation, or socio-demographic status of non-participants for comparison, but level of uptake by professional role was deemed to be representative of the hospital HCW population in both hospitals. The absence of real time genomic sequencing data precludes identifying the role of hospital outbreaks in influencing the overall seroprevalence, as well as drawing any definite conclusions regarding attributable risk to the workplace versus the community for HCW.

Detection of anti-spike antibody in conjunction with a self-reported history of vaccination was considered to be as a response to vaccination. It is possible that some of these participants may have detectable anti-spike antibody related to natural infection; these were not counted when assessing overall seroprevalence of presumed past infection, and therefore this overall seroprevalence may be an underestimate. Seroprevalence could also be underestimated because recent infection could be missed as several days are required for seroconversion of SARS-CoV-2 infection. Finally, some false negatives and false positives are expected with all laboratory tests. When the estimate of seroprevalence is adjusted using the manufacturer's stated specificity of 99.8% and sensitivity of 99.5%, (the seroprevalence does not change (18%; 95% CI 17% - 19%).

5.8 Conclusion and Recommendations

This study is a unique comparison between two hospitals in areas of differing community incidence over time, in which IPC measures were the same. The overall seroprevalence of antibodies to SARS-CoV-2 of 21% in SJH and 13% in UHG reflect the difference in community incidence in each area and the difference in rates of

confirmed infections among the HCW of each hospital. Risk was higher in the hospital situated in a higher density area with higher community incidence throughout the COVID-19 pandemic. The rise in seroprevalence from 15% to 21% in SJH in and from 4.1% to 13% in UHG between October 2020 and April 2021 reflect the magnitude of the third wave of the pandemic in each location. This data compounded the findings of PRECISE 1, highlighting the local community incidence as one of the most significant risk factors for acquisition of COVID-19 infection in HCW. It is also likely that social and demographic factors, and local work practices influenced the overall seroprevalence. The lack of real time genomic sequencing precludes identifying the role of hospital outbreaks in influencing the overall seroprevalence, as well as drawing conclusions regarding attributable risk to the healthcare environment versus the community or household.

Other risk factors common to both PRECISE 1 and 2 included demographic risk factors (younger age group, male and Black or Asian ethnic group) and work-place related risk factors representing close patient contact, including the role of HCA. We identified living with other HCW as an independent risk factor for seropositivity in both studies; to the best of our knowledge there is no other published literature commenting specifically on this risk factor. The other risk factors that we identified are consistent with the published literature.

The comparison between PRECISE 1 and 2 highlights similar features throughout the pandemic, but also the changing epidemiology with different waves. The main differences in the findings of PRECISE 1 and PRECISE 2 were related to role, ethnicity and level of education, showing that certain risks may change with different waves of the pandemic. The large increase in the seropositivity among general support staff and higher seroprevalence among doctors in UHG compared to SJH are difficult to fully explain and may relate to social factors. While both studies highlighted minority ethnic groups as more at risk, the group highlighted in each study was different. Level of education, which has been shown internationally to play a role, emerged as a new significant finding in PRECISE 2. This, coupled with the findings of risk related to ethnicity, may suggest that, even a year and a half into the pandemic, some groups may not have been adequately reached by messaging and education, and ongoing efforts need to be made in this regard.

The degree of previously undiagnosed infections highlights the need for ongoing universal adherence to infection control guidance including the use of appropriate PPE in the hospital setting, as well as the importance of early case detection. It is essential that HCW have easy access to testing, even with mild or no symptoms, and even in the post vaccination setting. (82). We recommend ongoing risk assessment in the setting of a hospital outbreak, and where indicated, screening of HCW, including those without symptoms, and including those who are vaccinated.

Ninety percent of those who were anti-body positive (anti-N) in October 2020 and took part in April 2021 were remained positive (anti-N). Further research is needed to understand the anti-N seroconversion following natural infection in vaccinated individuals to inform optimal assessment of seroprevalence in vaccinated cohorts.

The antibody response to vaccination is reassuring, however we did show breakthrough infection in a small minority of fully vaccinated participants; further studies are needed to correlate serological response with functional immunity. Formal vaccine effectiveness studies are needed to monitor how effective COVID-19 vaccines are in hospital HCW and to estimate duration of protection from infection, particularly with the ongoing emergence of variants of concern. With emerging evidence of reduction in transmission from vaccinated individuals, the authors strongly endorse immediate vaccination of all HCW. Messaging to HCW regarding the role and limits of vaccination need to be clear and should include the ongoing risk of infection and transmission. Ongoing adherence to all infection prevention and control standards in the healthcare setting and household are paramount. Easy access to testing of HCW with symptoms (including mild symptoms and including those who are vaccinated) and in the setting of close contact with a confirmed case of COVID-19 infection should continue, and vaccinated HCW with PCR-confirmed COVID-19 infection should be actively assessed to advance understanding of the reasons for breakthrough infection. This should include whole genome sequencing (WGS) of the virus from HCW with breakthrough infection and/or of virus from index cases identified by follow-up.

Chapter 6: Changes in individual linked serostatus over a six-month time period; a longitudinal study

6.1 Introduction

6.2 Methods

6.2.1 Statistical methods

6.3 Results

6.3.1 Seroreversion

6.3.2 Antibody retention

6.3.3 Seroconversion

6.4 Discussion and Recommendations

6.4.1 Sustained antibody response over six months

6.4.2 Seroreversion and its association with symptom severity and time since PCR positivity

6.1 Introduction

The specificity of SARS-Co-V-2 antibodies as an indicator of past COVID-19 infection is high (27), however, sensitivity wanes over time, (33) (34), with different antibodies waning at different rates, as detailed in Chapter 4. Some studies have shown a link between persistence of antibodies and severity of symptoms and type of symptoms (139), but antibody waning is likely to be influenced by the interplay of many other factors, including personal immunological factors, which are not as clearly delineated. Future epidemiological studies using SARS-Co-V-2 antibodies as a marker of past infection rely on improved understanding of duration of antibody responses in any given individual or population.

In this study, we aimed to assess antibody persistence over the six-month period between PRECISE 1 and PRECISE 2 for those HCW who participated in both studies. We assessed for any clinical characteristics that were associated with antibody loss. We also looked at the rate of seroconversion of those who were antibody negative in PRECISE 1.

6.2 Methods

In the PRECISE 2 questionnaire participants were asked if they had participated in PRECISE 1. These participants were matched using the unique identifier number generated by the online questionnaire (68). For those who did not match using this unique identifier (e.g. because they created a new online account rather than re-using their previously created account from PRECISE 1), we used the laboratory MRN generated by the Swiftqueue blood booking system to match their results (69). Similarly, for those who created a new blood booking account and did not match via laboratory MRN, name and surname, DOB and phone number were used to match the remaining participants; two/three of these data points were required to be considered a match. This was done using the Vlookup function in excel. For the purposes of this longitudinal part of the study, to facilitate direct comparison of individuals who participated in both studies, antibody positivity was defined as a detectable antibody on the Roche Elecsys anti-N total antibody assay, which was the assay used in both studies. This excluded 16 participants who participated both times and had detectable anti-N antibodies on the Abbott Architect IgG assay which was also used in PRECISE 1 but not used in PRECISE 2. (These 16 participants did not have detectable anti-N antibodies on the Roche assay in PRECISE 1).

6.2.1 Statistical methods

The chi-square distribution was used to compute confidence intervals (CI) and p-values. Binary logistic regression was used to calculate relative risks along with their 95% CIs, to assess the association between SARS-CoV-2 seroreversion and symptom severity adjusted for time since PCR positive test. PCR positivity refers to the date of most recent PCR positive test, and accounts for participants who were positive in the first phase and had a subsequent PCR confirmed infection (indicating re-infection) (n=5 matched participants).

All analysis was conducted in Stata 15.1 (StataCorp LCC. 2019. College Station, TX 77845: USA).

6.3 Results

In total, 3,313 qualifying study participants participated in both phases of the PRECISE study (demographics of these 3,313 are shown in Table 6A, Appendix). Of those, 18% (595/3313) were ever seropositive in October 2020 or April 2021; 11% (360/3313) were positive in October, 17% (560/3313) were positive in April, and 9.8% (325/3313) were seropositive in both studies (Table 16).

Table 16. Comparison of individual serological results in PRECISE 1 (October 2020) and PRECISE 2 (April 2021), Roche (anti-N) total antibody assay

Serological result PRECISE 1	**Serological result** PRECISE 2		
	Negative	**Positive**	**Total**
Negative	2718	235	2953
Positive	35	325	360
Total	2753	560	3313

6.3.1 Seroreversion

Of the 360 participants that were seropositive in PRECISE 1, 9.7% (n=35) experienced seroreversion (loss of previously documented antibody), i.e. they were seropositive in PRECISE 1 (October 2020) but seronegative in PRECISE 2 (April 2021). The proportion and 95% confidence intervals (CI) of participants that experienced seroreversion by demographic characteristics are shown in Table 17a. There were no participant characteristics that were significantly associated with seroreversion.

The proportion of participants that experienced seroreversion increased with decreasing symptom severity, but this was not significant (Table 17b). The proportion was 15% among those who had never experienced symptoms of COVID-19, 9.9% among those who had experienced mild symptoms, 8.3% among those who had experienced significant (moderate) symptoms, and 5.3% among those who had experienced severe symptoms.

Of the 360 participants who experienced seroreversion, 228 (63%) reported having had a previous PCR confirmed infection. The interval between date of previous PCR confirmed infection and phase 2 of the study ranged from 2 months to 13 months. There were no participants with seroreversion whose interval between PRECISE 2 and PCR was ≤10 months, this should be interpreted with caution due to low numbers in this group. Seroreversion was 15% for those whose interval was 13 months, 5.2% for those whose interval was 12 months,

and 16% whose interval was 11 months (confidence intervals overlap) (Table 17b). Seroreversion (and 95% CIs) by symptom severity among those previously PCR positive is shown in Figure 5.

When symptom severity was adjusted by time since PCR confirmed infection, a similar trend was observed (the highest risk of seroreversion was observed for those that had not previously experienced COVID-19 symptoms; aRR 2.0 (95% CI 0.2-25), but this was not significant (p=0.439) (Table 18a). Time since PCR positivity adjusted for symptom severity is shown in Table 18b and was also not significant.

Table 17a Proportion and 95% CI of PRECISE-1 participants that experienced seroreversion (October 2020 to April 2021), by demographic characteristics

Participant characteristics		**Total**	**Experienced seroreversion**		
		N	**n**	**% (95% CI)**	**p-value***
		360	35	9.7 (6.9-13)	
Age groups	18-29	96	9	9.4 (4.4-17)	0.483
	30-39	110	14	13 (7.1-20)	
	40-49	79	8	10 (4.5-19)	
	50-59	59	4	6.8 (1.9-16)	
	≥60	16	0	-	
Sex	Female	271	31	11 (7.9-16)	0.055
	Male	89	4	4.5 (1.2-11)	
Ethnicity	Irish	242	25	10 (6.8-15)	0.324
	Any other white background	45	7	16 (6.5-29)	
	Any Asian background	64	3	4.7 (1.0-13)	
	Any African or black background	8	0	-	
	Other	1	0	-	
Country of birth	Ireland	232	25	11 (7.1-15)	0.304
	United Kingdom	23	3	13 (2.8-34)	
	India	29	2	6.9 (0.8-23)	
	Philippines	31	1	3.2 (0.1-17)	
	Poland	6	2	33 (4.3-78)	
	USA	3	0	-	
	Other	36	2	5.6 (0.7-19)	
Education	Primary	2	0	-	0.193
	Secondary	32	2	6.3 (0.8-21)	
	Third level	181	13	7.2 (3.9-12)	
	Post-graduate	145	20	14 (8.6-20)	
Role	Admin	30	3	10 (2.1-27)	0.319
	Medical/dental	50	7	14 (5.8-27)	
	Nursing/ midwifery	184	15	8.2 (4.6-13)	
	Allied health	47	7	15 (6.2-28)	
	General support	19	3	16 (3.4-40)	
	Health care assistant	27	0	-	
	Other	3	0	-	
Lives with	Alone	18	2	11 (1.4-35)	0.929
	With others	341	33	9.7 (6.8-13)	
	Missing	1	0	-	
Lives with HCW	Yes	147	14	8.8 (4.8-15)	0.625
	No	206	21	10 (6.5-15)	
	Missing	7	0	11 (0.3-48)	
Daily contact with COVID-19 patients	Contact with COVID-19 patients	85	11	13 (6.6-22)	0.420
	Contact with patients without COVID-19	214	20	9.3 (5.8-14)	
	No patient contact	61	4	6.6 (1.8-16)	

*Calculated using the chi-square test

Table 17b Proportion and 95% CI of PRECISE-1 participants that experienced seroreversion (October 2020 to April 2021), by previous symptoms and by date of previous PCR confirmed infection

Participant characteristics		**Total**	**Experienced seroreversion**		
All		**N**	**n**	**% (95% CI)**	**p-value***
		360	35	9.7 (6.9-13)	
Previous COVID-19 symptoms	No symptoms	47	7	15 (6.2-28)	0.199
	Had symptoms	313	28	9 (6.0-13)	
Severity of symptoms	No symptoms	47	7	15 (6.2-28)	0.540
	Mild symptoms	162	16	9.9 (5.8-16)	
	Significant symptoms	132	11	8.3 (4.2-14)	
	Severe (hospitalised)	19	1	5.3 (0.1-26)	
Previous positive COVID-19 PCR test?	No	132	15	11 (6.5-18)	0.424
	Yes	228	20	8.8 (5.4-13)	
Number of months since most recent PCR positive test	1	0	0	-	0.349
	2	1	0	-	
	3	1	0	-	
	4	0	0	-	
	5	0	0	-	
	6	5	0	-	
	7	9	0	-	
	8	1	0	-	
	9	0	0	-	
	10	4	0	-	
	11	50	8	16 (7.2-29)	
	12	116	6	5.2 (1.9-11)	
	13	41	6	15 (5.6-29)	
Symptoms at time of most recent PCR test	No symptoms	17	2	12 (1.5-36)	0.917
	Mild symptoms	83	8	9.6 (4.3-18)	
	Significant (moderate)	111	9	8.1 (3.8-15)	
	Severe (hospitalised)	17	1	5.9 (0.1-29)	

*Calculated using the chi-square test

Table 18a Association between symptom severity and seroreversion (October 2020 to April 2021) among PRECISE participants, controlled for time since PCR confirmed infection

Participant characteristics		**Adjusted relative risk**	
		% (95% CI)	**p-value**
Symptoms at time of previous positive PCR test	No symptoms	2.0 (0.2-25)	0.439
	Mild symptoms	1.7 (0.2-13)	0.590
	Significant (moderate) symptoms	1.4 (0.2-10)	0.733
	Severe (hospitalised)	Ref.	

Table 18b Association between time since PCR confirmed infection and seroreversion (October 2020 - April 2021) among PRECISE participants, controlled for symptom severity

Participant characteristics		Adjusted relative risk	
		% (95% CI)	p-value
Number of months since most recent PCR positive test	≤11	Ref.	
	12	0.4 (0.2-1.2)	0.101
	13	1.1 (0.4-2.9)	0.843

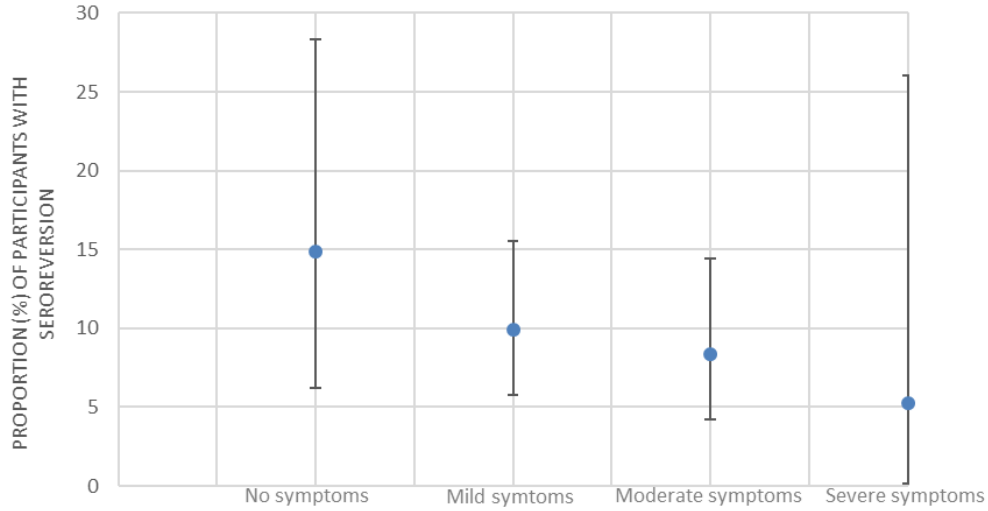


Figure 5 Proportion of PRECISE participants that experienced seroreversion between phase 1 (October 2020) and phase 2 (April 2021), by COVID-19 symptom severity

6.3.2 Antibody retention

Ninety percent (325/360) of those who were seropositive in phase 1 were also seropositive in phase 2. Of those, five reported having had PCR confirmed infection on two separate occasions, three had their second episode in October 2020 and two had their second episode in January 2021. The remaining 98% (320/325) did not report having had a PCR confirmed infection since October 2020 and are presumed to have maintained antibody positivity between both phases of the study.

6.3.3 Seroconversion

Of the 560 matched participants that were seropositive in phase 2, 235 were seronegative in PRECISE 1, i.e., they seroconverted between October 2020 and April 2021. Characteristics of those 235 participants are shown in Table 6B (Appendix).

6.4 Discussion and Recommendations

Among the 3,313 participants that were common to both PRECISE 1 and 2, 18% (595/3313) were ever seropositive; 11% (360/3313) in PRECISE 1 and 17% (560/3313) in PRECISE 2, for a seroconversion of 8% between October 2020 and April 2021, which was in keeping with the increased seropositivity from October to April in the overall cohort.

6.4.1 Sustained Antibody Response over six-months

Ninety percent of participants who were common to both studies, and were seropositive in PRECISE 1, still had antibodies at the time of PRECISE 2. As discussed in Chapter 4, the duration of antibody response in the literature is varied (35) (38), and further studies are still needed to directly correlate sustained antibody positivity with protection from re-infection, especially as the pandemic progresses and further VOCs emerge. This has implication at individual level in terms of potential protection from past infection, and at public health level in terms of the use of serological testing in determining population seroprevalence.

6.4.2 Seroreversion and its association with symptom severity and time since PCR positivity

Almost 10% of those who were seropositive in October 2020 seroreverted by April 2021. Although serological studies are still our best means of determining true rates of past infection in any given population, this highlights the limitations of its use, particularly as the pandemic progresses. As previously discussed, further studies are needed to delineate the exact relationship between seropositivity and protection from re-infection (with either the same or a different variant) but messaging regarding the loss of antibodies over time post natural infection may also be a useful adjunct in addressing vaccine hesitancy. No host factors in our study were significantly associated with antibody waning, however we did not collect information on host determinants such as underlying medical conditions, which may impact seroreversion. Future studies that include such information would add valuable understanding to this field.

While findings were not statistically significant, the data showed a weak trend for severity of symptoms being associated with retained antibody positivity, which would be in keeping with the published literature (139). Increasing time since positive PCR also showed a trend towards loss of antibody positivity, however the number of participants with a recent positive PCR was too low for any conclusion to be drawn. We previously showed this trend for a different assay (Abbott IgG anti-N assay) in our comparison of laboratory assays that is discussed in Chapter 4, but we did not see a waning on the Roche assay by this stage (140). It may be that by October 2020, the infections that were most distant in time from serological testing had happened six-seven months prior to serological testing; with this longitudinal matching to April 2021 serology, we now have infections that are

more distant in time (>10 months prior to serological testing) and therefore may be picking up a trend that could be more significant with a larger dataset over time. This result highlighted some of the advantages of incorporating a longitudinal component into this research, as opposed to the cross-sectional components. Further studies are needed to determine if this is the case for this assay, as well as to compare different serological assays as the pandemic progresses, to determine if antibodies eventually wane on all serological tests, including panantibody assays such as that used for this component of our study. |Further research is also needed to advance understanding of antibody neutralisation in relation to host immunity and protection with increasing viral diversity.

Chapter 7: Summary, Conclusion and Recommendations

7.1 Summary

7.2 Conclusion and Recommendations

7.2.1 Limitations

7.2.2 Lessons learned

7.2.3 Next Steps

7.2.4 Learning for future pandemics

7.1 Summary

Hospital healthcare workers (HCW) are at increased risk of contracting COVID-19 infection. The degree of increased risk is influenced by the local community seroprevalence, by working role within the hospital, and by social and demographic factors. The HCW seroprevalence was six times higher than community seroprevalence following the first wave of the pandemic in Ireland and rose further with subsequent waves. At both points in time during this study which involved cross-sectional studies in October 2020 and April 2021, the SARS-CoV-2 seropositivity was higher in the Dublin hospital, reflecting the higher community seroprevalence in the more densely populated capital city. By April 2021, at the end of the third wave of the pandemic in Ireland, 21% of staff in SJH and 13% of staff in UHG were seropositive, indicating infection with COVID-19 at least once. Working in close proximity to patients carried a higher risk of COVID-19 infection at both points in time during the study. The role of healthcare assistant was highlighted at both points in time as a risk factor, carrying up to double the risk of seropositivity compared to administration staff.

In terms of demographic factors, being male, young and of Asian or Black ethnicity were also linked to seropositivity, all findings that corroborate the published literature (84). Living with other healthcare workers was also shown to be a risk factor for seropositivity, highlighting the potential role of household transmission between HCW. Again, this was higher in Dublin, where smaller living spaces and higher accommodation costs likely mean smaller shared living spaces.

The comparison between the two studies highlights similar features throughout the pandemic, but also the changing epidemiology with different waves. To the best of our knowledge this was the first published study of its kind to compare epidemiology in the same population at two points in time during the pandemic. The main differences in the findings between October 2020 and April 2021 were related to role, ethnicity and level of education, showing that certain risks may change with different waves of the pandemic. There was a large increase in the seropositivity among general support staff and higher seroprevalence among doctors in UHG compared to SJH in April 2021, which may relate to social factors. While both studies highlighted minority ethnic groups as more at risk, the group highlighted in each study was different; Asian ethnicity in October 2021 and Black ethnicity in April 2022. Level of education, which has been shown internationally to play a role (137), emerged as a new significant finding in April 2021; those with a lower level of education were more likely to be seropositive.

In both cross-sectional studies, there was a high rate of previously undiagnosed infection, i.e. seropositive individuals who were not previously aware of ever having been infected with COVID-19. Although this decreased from 39% in October 2020 to 26% in April 2021, still a quarter of infections went undiagnosed, despite easy access to testing on site in both locations, and despite the majority of these having had symptoms

consistent with COVID-19 infection at some stage. The majority performed roles involving close patient contact, and therefore were assumably aware of the risks of infection acquisition, had access to PPE recognised the symptoms associated with infection, and the availability of free onsite testing.

The study assessing antibody production post vaccination was very encouraging, showing both high uptake of vaccination among HCW and demonstrating that 100% of fully vaccinated participants had anti-spike antibodies, the type of antibody produced in response to vaccination, at 3 months after the start of the vaccination programme in Ireland. Twenty-three of these had had breakthrough COVID-19 infection, representing 0.6% of all fully vaccinated participants. This was significantly different to the 13% of partially vaccinated participants who experienced a confirmed infection post vaccination.

We studied antibody persistence in two ways in this research programme, described in Chapters 4 and 6. First, we compared antibody positivity on two different platforms in those with a known prior infection, to see how long the antibody persisted post positive PCR. Secondly, we compared the results of those who were antibody positive in October 2021 and participated again in April 2021, to see if they remained seropositive.

The comparison of laboratory assays detailed in Chapter 4 highlighted the fact that not all assays perform the same and serves as a reminder that the results of seroprevalence studies are only as good as the sustained sensitivity and specificity of the assays used. Using the subgroup of participants with previously confirmed COVID-19 infection, we were able to compare the two assays used for the study, and while the Roche assay detected 95% of these infections and had sustained positivity up to 33 weeks after infection, only 41% of these participants tested positive on the Abbott assay. The decline in Abbott positivity starting at week 21/day 150 after confirmed infection.

The final chapter (chapter 6) evaluating sustained antibody response in participants known to be antibody positive in October 2021 showed that 90% of these participants remained antibody positive six months later. Of the 10% who seroreverted, there were no characteristics that were statistically significantly associated with seroeversion, but there was a trend towards longer distance in time from the confirmed infection being associated with loss of antibody. There was also a weak trend towards more severe symptoms being associated with retained antibody positivity.

7.2 Conclusion and Recommendations

These studies are a unique comparison between two hospitals in areas of differing community incidence over time. The overall seroprevalence of antibodies to SARS-CoV-2 of 21% in SJH and 13% in UHG reflects the difference in community incidence in each area and the difference in rates of confirmed infections among the

HCW of each hospital. Risk was higher in the hospital situated in a higher density area with higher community incidence throughout the COVID-19 pandemic. The rise in seroprevalence from 15% to 21% in SJH in and from 4.1% to 13% in UHG between October 2020 and April 2021 reflects the magnitude of the third wave of the pandemic in each location. The data from both cross-sectional studies highlight the local community incidence as one of the most significant risk factors for acquisition of COVID-19 infection in HCW. It is also likely that social and demographic factors, and local work practices influenced the overall seroprevalence. The lack of real time genomic sequencing precludes identifying the role of hospital outbreaks in influencing the overall seroprevalence, as well as drawing conclusions regarding attributable risk to the healthcare environment versus the community or household.

There were many risk factors that remained the same from October 2020 to April 2021, including demographic risk factors and work-place related risk factors representing close patient contact. We identified living with other HCW as an independent risk factor for seropositivity in both studies; to the best of our knowledge there is no other published literature commenting specifically on this risk factor. The other risk factors that we identified are consistent with the published literature.

The comparison between the two points in time highlights the changing epidemiology of the pandemic with different waves. The main differences in the findings of October 2020 and April 2021 were related to role, ethnicity and level of education, showing that certain risks may change with different waves of the pandemic. The findings of risk related to level of education and ethnic group may suggest that, even a year and a half into the pandemic, some groups may not have been adequately reached by messaging and education, and ongoing efforts need to be made in this regard.

The degree of previously undiagnosed infections at both points in time highlights the need for ongoing universal adherence to infection control guidance including the use of appropriate PPE in the hospital setting, as well as the importance of early case detection. It is essential that HCW continue to be encouraged to test, and have easy access to testing facilities, even with mild symptoms and even in the post vaccination setting. (82). We recommend ongoing risk assessment in the setting of a hospital outbreak, and where indicated, screening of HCW, including those without symptoms, and including those who are vaccinated.

The antibody response to vaccination is reassuring, however we did show confirmed infection in a small minority of fully vaccinated participants, all of whom had an appropriate serological response to vaccination; further studies are needed to correlate anti-S serological response with functional immunity. Formal vaccine effectiveness studies are needed to monitor how effective COVID-19 vaccines are in hospital HCW and to estimate duration of protection from infection, particularly with the ongoing emergence of variants of concern. As a result of this research both study sites are now involved in a vaccine efficacy study. With emerging evidence of reduction in transmission from vaccinated individuals (137), we recommend ongoing vaccination of all HCW as a priority. Messaging to HCW regarding the role and limits of vaccination need to be clear and

should include the ongoing risk of infection and transmission, and the need to continue to test and isolate in the presence of symptoms. Adherence to all infection prevention and control standards in the healthcare setting should continue in the post-vaccination era, and vaccinated HCW with PCR-confirmed COVID-19 infection should be actively assessed to advance understanding of the reasons for breakthrough infection. This should include whole genome sequencing (WGS) of the virus from HCW with breakthrough infection and/or of virus from index cases identified by follow-up.

Assessment of vaccinated cohorts now relies on anti-N antibody detection, to differentiate between antibodies produced by natural infection and antibodies produced by vaccination. In our study 90% of those who were anti-N positive in October 2021 remained anti-N positive in April 2021. No significant risk factors were identified to be associated with earlier seroeversion, perhaps due to the smaller numbers in this subgroup. Further research is needed to understand the anti-N seroconversion and seroeversion (timing of, and risk factors for) in both vaccinated and unvaccinated individuals, as well as to understand how anti-N antibody positivity relates to immunity, especially in light of emerging VOCs.

In terms of testing platforms, our study findings suggest that, of the two assays directed at anti-N antibodies (Roche and Abbott), Roche is better suited to future population-based serological studies, due to maintained detection of total antibodies up to at least seven months after natural infection with SARS-CoV-2. While we demonstrate good performance of Roche anti-nucleocapsid assay at extended time points, it remains to be seen whether, and after how long, the detection of antibodies on this platform would also wane. Further studies are also needed to determine whether all panantibody assays, such as Roche, perform superiorly to IgG antibody assays, such as Abbott, or whether this finding was specific to the exact bran(84)d of assays used in this study. The anti-spike assay (Wantai) performed very well on the subset of samples analysed, but further studies are needed to show if it maintains its sensitivity compared to Roche on an unbiased selection of samples. Furthermore, with the advent of vaccination, anti-spike only assays are no longer as useful for assessing for past natural infection in a largely vaccinated cohort; anti-N antibody assays are needed to differentiate between serology related to vaccination and serology related to natural infection.

This comparison of assay performance adds to the growing literature on serological assays for population-based studies. Further data comparing antibody assays for the detection of antibodies to SARS-CoV-2 are needed to guide the most appropriate use of certain assays in any given situation, especially where use of dual or multiple assays is not feasible or affordable. Assays, particularly anti-N antibody assays, with prolonged sensitivity are likely to be more valuable as the pandemic continues.

In April 2021, 90% of participants who were antibody positive in October 2020 remained seropositive. As discussed above, the duration of antibody response is varied, both in our study and in the published literature (35) (38), and further studies are still needed to delineate the relationship between serological response, and sustained response, and functional immunity for repeat infection protection from re-infection, especially as the

pandemic progresses and further VOCs emerge. This needs to be assessed both for serological response to vaccination, and to natural infection. This has implication at individual level in terms of potential protection from past infection, and at public health level in terms of the use of serological testing in determining population seroprevalence.

7.2.1 Limitations

The individual sub-study limitations are described in detail in the relevant chapters. The overall research programme had some limitations, which came to light as the pandemic evolved, subsequent sub-studies post-dating this research evolved with the pandemic. The research programme was not designed as a cohort study, but rather as two cross-sectional studies, with a longitudinal link between the two. This meant that linkage of data between the two studies was laborious. More importantly, as the research continued, the need for a cohort study was recognised, and PRECISE 3, the next sub-study after the research described here, is designed as such, and is ongoing. Linkage of all data between PRECISE 1, 2, and 3, may have been made easier had the study design been a cohort study from the start, however this would have impacted other key factors such as timeliness, uptake and ongoing participation. At design and implementation of PRECISE 1 there was not a clarity to the expected duration of pandemic. We used an online platform to provide the study questionnaire, prioritising easy and quick participation in the setting of national lockdown, however we recognise that this may have excluded some HCW . All efforts were made to mitigate this, as detailed above. The programme emerged with pandemic with limited time available to commence the work that required full ethical approval. This included a review in detail of some aspects of the study design and initial questionnaire, therefore we collected limited clinical data but did not prioritise data on co-morbidities/ immunosuppression. In retrospect, this data may have helped our understanding of vaccination response, re-infection and severity of infection. Initially antibody testing on the Roche platform was less sensitive than currently used tests, but the study had the agility to compensate for this with a change in testing platform in response to emerging data. The sub-studies described here were not designed to study re-infection in any detail, and there was no correlation with WGS of isolates, however this is now being done in PRECISE 3 which is ongoing.

7.2.2 Lessons learned

This was my first experience as Principal Investigator for a national research programme, involving multiple studies conducted in two separate locations. A huge amount was learned both by me and by the research team. One of the notable features of this research was that much of it was conducted during a period of governmental restrictions due to COVID-19. This was a new playing field for clinicians as well as researchers, and I learned

valuable lessons about how to manage teams remotely (I was based at one study site, and only manged to get to the other study site twice during Level 5 restrictions, yet I was responsible for the core study teams at both sites and the day to day running of the study). Promoting the study widely throughout both hospitals had to be done using innovative means, as described in methods section of Chapter 3, as most in-person meetings were cancelled. Ensuring the phlebotomy component of the study complied with social distancing regulations was another challenge; we tried to balance this with the inclusion of walk-in clinics and un-planned visits to maximise uptake.

Key lessons were learned pertaining to data management, data sharing and data analysis. Due to the remote working conditions, we discovered after the data collection for PRECISE 1 that there were slight differences in software versions and data storage between the sites that complicated the merging of the data, and a key lesson learned was to ensure all data collection was on same platform with identical software version, and ideally all data analysis to be conducted in the same software programme, so that data scripts could be easily shared with any changeover of the person conducting the analysis.

Data dissemination was a vital component of this research programme. We wanted to make our results available as close to real time as possible, to help national decision makers, as well as the wider international community dealing with the rapidly evolving situation. This was difficult to achieve with a study steering group all needing to sign off any reports, and this was particularly clear over the Christmas period of 2020; the lab results for PRECISE 1 became available in the second week of December, so that timing was such that a basic analysis fell over the Christmas period, and I wanted to get the report out prior to the commencement of vaccination in early January. In future, I would agree among the group that sign off by a sub-set of nominated members could be deemed sufficient for preliminary reports, in order to not incur delays. I think that better time-line planning would also have helped this situation. However, part of the timing of this was due to the flexibility incorporated into the study – in late November we added serological testing on a second platform due to newly published literature regarding the sensitivity of the test we had used.

This research programme was designed rapidly, in an emerging situation which needed a quick response. This meant that some aspects of its development felt rushed, and while the national steering group and the lead site investigators made themselves wholly available to support the rapid development of the programme, one of the factors that we overlooked at the outset was a plan for data analysis. I designed the questionnaire for the first study myself and circulated it to the steering group and a selection of lay people for testing. I also did the basic analysis myself, but for advanced analysis I set up a data subgroup. This sub-group should have been set up at the outset of the study, rather than when the data became available. This team should have been involved in the design of the questionnaire, in order to control the type of data that would then be available to them. There were no major issues with the resulting data, as seen by very minimal changes for PRECISE 2 (for which the data sub-

group were involved from the outset) but in principle this would have been the correct order of conducting things and would have alleviated a lot of stress from the data analysis for the first study.

7.2.3 Next Steps

As a consequence of this research, our research consortium made a call for several further studies. Firstly, a longitudinal cohort of Irish HCW is being studied (PRECISE 3,4 and 5) for breakthrough infection, host determinants of disease, and vaccine response, with linkage to results of PRECISE 1 and 2 for access to baseline serology, prior to and post vaccination. Secondly, our research highlighted the need for formal VE studies, and due to the PRECISE research programme our two study sites were well positioned to partake in the European Centre for Disease Control (ECDC) Vaccine Effectiveness, Burden and Impact Studies (VEBIS), which are ongoing (141).Thirdly, we called for further studies on neutralising antibodies, which are being conducted as part of PRECISE 5. In the fourth instance, we advocated for emerging diagnostics such as real-time WGS, which is now in place systematically. In the fifth instance, we advocated for extension of research to other respiratory viruses, and influenza is now included in VEBIS (142). And finally, we are still advocating for dedicated HCW epidemiological units in hospitals nationally.

7.4.4 Learnings for future pandemics

The COVID-19 pandemic has a different impact on locations worldwide, resulting in a combination of universally shared challenges and regional-specific challenges. One key learning point, however, is shared by all; we were not prepared. As the pandemic has evolved, we have learned from our mistakes, or from the mistakes of others, and it is imperative that we take this learning into account in our preparation for future pandemics. This research has provided, and the PRECISE research consortium continues to provide, valuable information for consideration in such planning at national and international level. We know now how pertinent it is to consider the role of hospital/HCW epidemiology in local spread of infection. As has been the case with COVID-19 infection, HCW may have increased risk of infection, proportional to community incidence and patient exposure and inversely proportional to education level. This highlights the importance of IPC-related education targeting the different disciplines in healthcare provision. Social factors such as living with other HCW also need to be considered at the outset of a pandemic. The impact of asymptomatic infection, an issue that was contentious at the outset of the COVID-19 pandemic, as a contributor to nosocomial transmission of viral infections needs to be considered in any future pandemic. Reassuringly vaccine update was high among healthcare workers in the sub-studies presented here, however a decline in the uptake of booster doses raises the ongoing need for support and

dialogue with healthcare workers regarding vaccine acceptability. Research in a pandemic scenario is crucial and pathways should be in place to allow research programmes to be launched rapidly. This should include a centralised investigational ethics committee, as was present in Ireland, this facilitated the commencement of this study within the first six months of the pandemic. Preparedness should also include IT support for investigators and epidemiologists, preceding agreements with IT platforms to facilitate end-to-end solutions for data collection, and rapid GDPR-compliant data sharing agreements between institutions. We should continue to build strong links between clinicians, epidemiologists and national public health bodies.

References

1. European Centre for Disease Prevention and Control [Internet]. [cited 2020 Dec 5]. Available from: https://www.ecdc.europa.eu/en/geographical-distribution-2019-ncov-cases
2. Shah ASV, Wood R, Gribben C, Caldwell D, Bishop J, Weir A, et al. Risk of hospital admission with coronavirus disease 2019 in healthcare workers and their households: nationwide linkage cohort study. BMJ. 2020 Oct 28;m3582.
3. Karlsson U, Fraenkel CJ. Covid-19: risks to healthcare workers and their families. BMJ. 2020 Oct 28;m3944.
4. Richterman A, Meyerowitz EA, Cevik M. Hospital-Acquired SARS-CoV-2 Infection: Lessons for Public Health. JAMA. 2020 Dec 1;324(21):2155.
5. Nguyen LH, Drew DA, Graham MS, Joshi AD, Guo CG, Ma W, et al. Risk of COVID-19 among front-line health-care workers and the general community: a prospective cohort study. Lancet Public Health. 2020 Sep;5(9):e475–83.
6. Iversen K, Bundgaard H, Hasselbalch RB, Kristensen JH, Nielsen PB, Pries-Heje M, et al. Risk of COVID-19 in health-care workers in Denmark: an observational cohort study. Lancet Infect Dis. 2020;20(12):1401–8.
7. Lai X, Wang M, Qin C, Tan L, Ran L, Chen D, et al. Coronavirus Disease 2019 (COVID-2019) Infection Among Health Care Workers and Implications for Prevention Measures in a Tertiary Hospital in Wuhan, China. JAMA Netw Open. 2020 May 21;3(5):e209666–e209666.
8. Plebani M, Padoan A, Fedeli U, Schievano E, Vecchiato E, Lippi G, et al. SARS-CoV-2 serosurvey in health care workers of the Veneto Region. Clin Chem Lab Med CCLM [Internet]. 2020 Aug 26 [cited 2020 Dec 14];0(0). Available from: https://www.degruyter.com/view/journals/cclm/ahead-of-print/article-10.1515-cclm-2020-1236/article-10.1515-cclm-2020-1236.xml
9. Self WH, Tenforde MW, Stubblefield WB, Feldstein LR, Steingrub JS, Shapiro NI, et al. Seroprevalence of SARS-CoV-2 Among Frontline Health Care Personnel in a Multistate Hospital Network — 13 Academic Medical Centers, April–June 2020. MMWR Morb Mortal Wkly Rep. 2020 Sep 4;69(35):1221–6.
10. Korth J, Wilde B, Dolff S, Anastasiou OE, Krawczyk A, Jahn M, et al. SARS-CoV-2-specific antibody detection in healthcare workers in Germany with direct contact to COVID-19 patients. J Clin Virol. 2020 Jul;128:104437.
11. Garcia-Basteiro AL, Moncunill G, Tortajada M, Vidal M, Guinovart C, Jiménez A, et al. Seroprevalence of antibodies against SARS-CoV-2 among health care workers in a large Spanish reference hospital. Nat Commun. 2020 Dec;11(1):3500.
12. Jespersen S, Mikkelsen S, Greve T, Kaspersen KA, Tolstrup M, Boldsen JK, et al. SARS-CoV-2 seroprevalence survey among 17,971 healthcare and administrative personnel at hospitals, pre-hospital services, and specialist practitioners in the Central Denmark Region. Clin Infect Dis Off Publ Infect Dis Soc Am. 2020 Oct 3;
13. Steensels D, Oris E, Coninx L, Nuyens D, Delforge ML, Vermeersch P, et al. Hospital-Wide SARS-CoV-2 Antibody Screening in 3056 Staff in a Tertiary Center in Belgium. JAMA. 2020 Jul 14;324(2):195.
14. Houlihan C, Vora N, Byrne T, Lewer D, Heaney J, Moore DA, et al. SARS-CoV-2 virus and antibodies in front-line Health Care Workers in an acute hospital in London: preliminary results from a longitudinal study [Internet]. Infectious Diseases (except HIV/AIDS); 2020 Jun [cited 2020 Jul 24]. Available from: http://medrxiv.org/lookup/doi/10.1101/2020.06.08.20120584
15. Psichogiou M, Karabinis A, Pavlopoulou ID, Basoulis D, Petsios K, Roussos S, et al. Antibodies against SARS-CoV-2 among health care workers in a country with low burden of COVID-19. PloS One. 2020;15(12):e0243025.

16. Galanis P, Vraka I, Fragkou D, Bilali A, Kaitelidou D. Seroprevalence of SARS-CoV-2 antibodies and associated factors in health care workers: a systematic review and meta-analysis. J Hosp Infect. 2020 Nov 16;
17. Noor M, Haq M, Ul Haq N, Amin S, Rahim F, Bahadur S, et al. Does Working in a COVID-19 Receiving Health Facility Influence Seroprevalence to SARS-CoV-2? Cureus [Internet]. 2020 Nov 9 [cited 2020 Dec 14]; Available from: https://www.cureus.com/articles/44953-does-working-in-a-covid-19-receiving-health-facility-influence-seroprevalence-to-sars-cov-2
18. Yanes-Lane M, Winters N, Fregonese F, Bastos M, Perlman-Arrow S, Campbell JR, et al. Proportion of asymptomatic infection among COVID-19 positive persons and their transmission potential: A systematic review and meta-analysis. Serra R, editor. PLOS ONE. 2020 Nov 3;15(11):e0241536.
19. Rahimi F, Talebi Bezmin Abadi A. Challenges of managing the asymptomatic carriers of SARS-CoV-2. Travel Med Infect Dis. 2020 Oct;37:101677.
20. HPSC Surveillance for COVID-19 [Internet]. HPSC. 2021 [cited 2021 Jan 6]. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/surveillance/
21. WHO Coronavirus Disease (COVID-19) Dashboard. [cited 2020 Dec 12]; Available from: https://covid19.who.int/?gclid=Cj0KCQiA8dH-BRD_ARIsAC24umYzHWVDldt10mB3Jsupjm1-BYKBTfzTAA4mYl90FcjTWDHM00Z6qfUaAiH8EALw_wcB
22. Department of Tourism, Culture, Arts, Gaeltacht, Sport and Media Notification [Internet]. 2021. Available from: https://assets.gov.ie/125288/5ad28ed2-c8e7-4a3a-af0a-6a1516284bd4.pdf
23. HPSC Covid Cases in Ireland [Internet]. [cited 2021 May 12]. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/casesinireland/epidemiologyofcovid-19inireland/COVID19%20Daily%20infographic.pdf
24. Preliminary report of the results of the Study to Investigate COVID-19 Infection in People Living in Ireland (SCOPI): A national seroprevalence study, June-July 2020 [Internet]. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/scopi/SCOPI%20report%20preliminary%20results%20final%20version.pdf
25. COVID-19 cases in Healthcare Workers – 14-day report, Ireland, 27/12/2020 - 09/01/2021 [Internet]. [cited 2021 May 12]. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/surveillance/covid-19casesinhealthcareworkers/HCW_report_12%2001%202021_1.1%20website_final.pdf
26. Nearly one-fifth of Tallaght hospital staff found to have coronavirus antibodies. [cited 2020 Dec 12]; Available from: https://www.thejournal.ie/tallaght-hospital-staff-antibody-study-5245379-Oct2020/
27. Watson J, Richter A, Deeks J. Testing for SARS-CoV-2 antibodies. BMJ. 2020 Sep 8;m3325.
28. Walsh EE, Frenck RW, Falsey AR, Kitchin N, Absalon J, Gurtman A, et al. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. N Engl J Med. 2020 Dec 17;383(25):2439–50.
29. Vabret N, Britton GJ, Gruber C, Hegde S, Kim J, Kuksin M, et al. Immunology of COVID-19: Current State of the Science. Immunity. 2020 Jun;52(6):910–41.
30. Hanrath AT, Payne BAI, Duncan CJA. Prior SARS-CoV-2 infection is associated with protection against symptomatic reinfection. J Infect. 2020 Dec;S0163445320307817.
31. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis. 2020 Mar 28;ciaa344.
32. Post N, Eddy D, Huntley C, van Schalkwyk MCI, Shrotri M, Leeman D, et al. Antibody response to SARS-CoV-2 infection in humans: A systematic review. Mantis NJ, editor. PLOS ONE. 2020 Dec 31;15(12):e0244126.
33. Ibarrondo FJ, Fulcher JA, Goodman-Meza D, Elliott J, Hofmann C, Hausner MA, et al. Rapid Decay of Anti–SARS-CoV-2 Antibodies in Persons with Mild Covid-19. N Engl J Med. 2020 Sep 10;383(11):1085–7.

34. Patel MM, Thornburg NJ, Stubblefield WB, Talbot HK, Coughlin MM, Feldstein LR, et al. Change in Antibodies to SARS-CoV-2 Over 60 Days Among Health Care Personnel in Nashville, Tennessee. JAMA. 2020 Nov 3;324(17):1781.
35. Gudbjartsson DF, Norddahl GL, Melsted P, Gunnarsdottir K, Holm H, Eythorsson E, et al. Humoral Immune Response to SARS-CoV-2 in Iceland. N Engl J Med. 2020 Oct 29;383(18):1724–34.
36. Wajnberg A, Amanat F, Firpo A, Altman DR, Bailey MJ, Mansour M, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science. 2020 Dec 4;370(6521):1227–30.
37. National SARS-CoV-2 Serology Assay Evaluation Group. Performance characteristics of five immunoassays for SARS-CoV-2: a head-to-head benchmark comparison. Lancet Infect Dis. 2020 Dec;20(12):1390–400.
38. Muecksch F, Wise H, Batchelor B, Squires M, Semple E, Richardson C, et al. Longitudinal analysis of clinical serology assay performance and neutralising antibody levels in COVID19 convalescents [Internet]. Infectious Diseases (except HIV/AIDS); 2020 Aug [cited 2020 Dec 14]. Available from: http://medrxiv.org/lookup/doi/10.1101/2020.08.05.20169128
39. Lumley SF, Wei J, O'Donnell D, Stoesser NE, Matthews PC, Howarth A, et al. The duration, dynamics and determinants of SARS-CoV-2 antibody responses in individual healthcare workers. Clin Infect Dis. 2021 Jan 6;ciab004.
40. Summary of COVID-19 virus variants in Ireland Report prepared by HPSC and NVRL on 11/05/2021 [Internet]. HPSC; 2021 May [cited 2021 May 18]. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/surveillance/summaryofcovid-19virusvariantsinireland/Virus%20Variants%20report.pdf
41. EMA recommends first COVID-19 vaccine for authorisation in the EU [Internet]. 2020 [cited 2021 May 19]. Available from: https://www.ema.europa.eu/en/news/ema-recommends-first-covid-19-vaccine-authorisation-eu
42. EMA recommends COVID-19 Vaccine Moderna for authorisation in the EU [Internet]. 2021 [cited 2021 May 19]. Available from: https://www.ema.europa.eu/en/news/ema-recommends-covid-19-vaccine-moderna-authorisation-eu
43. EMA recommends COVID-19 Vaccine AstraZeneca for authorisation in the EU [Internet]. 2021 [cited 2021 May 19]. Available from: https://www.ema.europa.eu/en/news/ema-recommends-covid-19-vaccine-astrazeneca-authorisation-eu
44. EMA recommends COVID-19 Vaccine Janssen for authorisation in the EU [Internet]. 2021 [cited 2021 May 19]. Available from: https://www.ema.europa.eu/en/news/ema-recommends-covid-19-vaccine-janssen-authorisation-eu
45. National Immunisation Advisory Committee (NIAC) issues revised advice on the use of the AstraZeneca COVID-19 vaccine [Internet]. 2021 [cited 2021 May 12]. Available from: https://www.rcpi.ie/news/releases/national-immunisation-advisory-committee-niac-issues-revised-advice-on-the-use-of-the-astrazeneca-covid-19-vaccine/),
46. EMA; AstraZeneca's COVID-19 vaccine: EMA finds possible link to very rare cases of unusual blood clots with low blood platelets [Internet]. 2021 [cited 2021 May 12]. Available from: https://www.ema.europa.eu/en/news/astrazenecas-covid-19-vaccine-ema-finds-possible-link-very-rare-cases-unusual-blood-clots-low-blood
47. Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. The Lancet. 2021 Jan;397(10269):99–111.
48. Levine-Tiefenbrun M, Yelin I, Katz R, Herzel E, Golan Z, Schreiber L, et al. Decreased SARS-CoV-2 viral load following vaccination [Internet]. Infectious Diseases (except HIV/AIDS); 2021 Feb [cited 2021 Apr 13]. Available from: http://medrxiv.org/lookup/doi/10.1101/2021.02.06.21251283

49. Hall VJ, Foulkes S, Saei A, Andrews N, Oguti B, Charlett A, et al. Effectiveness of BNT162b2 mRNA Vaccine Against Infection and COVID-19 Vaccine Coverage in Healthcare Workers in England, Multicentre Prospective Cohort Study (the SIREN Study). SSRN Electron J [Internet]. 2021 [cited 2021 Apr 13]; Available from: https://www.ssrn.com/abstract=3790399
50. Bernal JL, Andrews N, Gower C, Stowe J, Robertson C, Tessier E, et al. Early effectiveness of COVID-19 vaccination with BNT162b2 mRNA vaccine and ChAdOx1 adenovirus vector vaccine on symptomatic disease, hospitalisations and mortality in older adults in England [Internet]. Infectious Diseases (except HIV/AIDS); 2021 Mar [cited 2021 Apr 13]. Available from: http://medrxiv.org/lookup/doi/10.1101/2021.03.01.21252652
51. Skelly DT, Harding AC, Gilbert-Jaramillo J, Knight ML, Longet S, Anthony Brown, et al. Vaccine-induced immunity provides more robust heterotypic immunity than natural infection to emerging SARS-CoV-2 variants of concern. [Internet]. In Review; 2021 Feb [cited 2021 May 19]. Available from: https://www.researchsquare.com/article/rs-226857/v1
52. Hoffmann M, Arora P, Groß R, Seidel A, Hörnich B, Hahn A, et al. SARS-CoV-2 variants B.1.351 and B.1.1.248: Escape from therapeutic antibodies and antibodies induced by infection and vaccination [Internet]. Molecular Biology; 2021 Feb [cited 2021 Apr 13]. Available from: http://biorxiv.org/lookup/doi/10.1101/2021.02.11.430787
53. Novavax COVID-19 Vaccine Demonstrates 89.3% Efficacy in UK Phase 3 Trial [Internet]. 2021 [cited 2021 Apr 15]. Available from: https://ir.novavax.com/news-releases/news-release-details/novavax-covid-19-vaccine-demonstrates-893-efficacy-uk-phase-3
54. Sahin U, Muik A, Derhovanessian E, Vogler I, Kranz LM, Vormehr M, et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. Nature. 2020 Oct 1;586(7830):594–9.
55. Lumley SF, O'Donnell D, Stoesser NE, Matthews PC, Howarth A, Hatch SB, et al. Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers. N Engl J Med. 2020 Dec 23;NEJMoa2034545.
56. St. James's Hospital Occupation Health Department.
57. University Hospital Galway, Occupational Department.
58. Epidemiology of COVID-19 Outbreaks/Clusters in Ireland Weekly Report Prepared by HPSC on 22nd March 2021 [Internet]. 2021 [cited 2021 May 18]. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/surveillance/covid-19outbreaksclustersinireland/COVID-19%20Weekly%20Outbreak%20Report_Week112021_WebVersion_final.pdf
59. Epidemiology of COVID-19 in Ireland - 14 day report prepared by HPSC on 24/01/2021 [Internet]. [cited 2021 Apr 19]. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/surveillance/covid-1914-dayepidemiologyreports/2021/january2021/COVID-19_14_day_epidemiology_report_20210124_Website.pdf
60. Epidemiology of COVID-19 in Ireland - 14 day report prepared by HPSC on 14/01/2021 [Internet]. 2021 [cited 2021 May 18]. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/surveillance/covid-1914-dayepidemiologyreports/2021/january2021/COVID-19_14_day_epidemiology_WEB%20report_20210114.pdf
61. Epidemiology of COVID-19 in Ireland - 14 day report prepared by HPSC on 16/04/2021 [Internet]. [cited 2021 Apr 19]. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/surveillance/covid-1914-dayepidemiologyreports/COVID-19_14_day_epidemiology_report_20210416_WEB.pdf
62. SARS-CoV-2 IgG Architect - Instructions for Use, FDA [Internet]. [cited 2020 Dec 14]. Available from: https://www.fda.gov/media/137383/download
63. ARCHITECT System SARS-COV-2IgG/lgM e- Assay CD-ROM - WW (excluding US) List number 6514-03. Information update 07th October 2021.

64. Elecsys® Anti-SARS-CoV-2 Immunoassay for the qualitative detection of antibodies (incl. IgG) against SARS-CoV-2 [Internet]. [cited 2020 Dec 14]. Available from: https://diagnostics.roche.com/global/en/products/params/elecsys-anti-sars-cov-2.html
65. WANTAI SARS-CoV-2 Ab ELISA - FDA [Internet]. Available from: https://www.fda.gov/media/140929/download
66. Overview of COVID-19 vaccination strategies and vaccine deployment plans in the EU/EEA and the UK [Internet]. [cited 2021 Jan 12]. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/Overview-of-EU_EEA-UK-vaccination-deployment-plans.pdf
67. Irish National COVID-19 Vaccination Strategy and Implementation Plan [Internet]. [cited 2021 Feb 12]. Available from: https://www.gov.ie/en/publication/bf337-covid-19-vaccination-strategy-and-implementation-plan/#
68. Castor EDC. (2021). Castor Electronic Data Capture. [online] Available at: https://castoredc.com. [Internet]. 2021. Available from: https://www.castoredc.com/
69. Swiftqueue booking platform [Internet]. Available from: https://www.swiftqueue.com/
70. Bryan A, Pepper G, Wener MH, Fink SL, Morishima C, Chaudhary A, et al. Performance Characteristics of the Abbott Architect SARS-CoV-2 IgG Assay and Seroprevalence in Boise, Idaho. McAdam AJ, editor. J Clin Microbiol. 2020 May 7;58(8):e00941-20, /jcm/58/8/JCM.00941-20.atom.
71. Crawford KHD, Dingens AS, Eguia R, Wolf CR, Wilcox N, Logue JK, et al. Dynamics of Neutralizing Antibody Titers in the Months After Severe Acute Respiratory Syndrome Coronavirus 2 Infection. J Infect Dis. 2020 Sep 30;jiaa618.
72. Elecsys® Anti-SARS-CoV-2 S Immunoassay for the quantitative determination of antibodies to the SARS-CoV-2 spike protein [Internet]. [cited 2021 May 20]. Available from: https://diagnostics.roche.com/global/en/products/params/elecsys-anti-sars-cov-2-s.html
73. Martin CA, Patel P, Goss C, Jenkins DR, Price A, Barton L, et al. Demographic and occupational determinants of anti-SARS-CoV-2 IgG seropositivity in hospital staff. J Public Health Oxf Engl. 2020 Nov 16;
74. Occupational Health Department data, SJH and UHG.
75. Sayampanathan AA, Heng CS, Pin PH, Pang J, Leong TY, Lee VJ. Infectivity of asymptomatic versus symptomatic COVID-19. The Lancet. 2021 Jan;397(10269):93–4.
76. HPSC Infection Prevention and Control Precautions for COVID-19 [Internet]. [cited 2021 Jan 12]. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/guidance/infectionpreventionandcontrolguidance/
77. HPSC General guide on management of COVID-19 outbreaks in the workplace [Internet]. [cited 2021 Jan 12]. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/guidance/outbreakmanagementguidance/Guidance%20of%20work%20place%20outbreaks.pdf
78. Rivett L, Sridhar S, Sparkes D, Routledge M, Jones NK, Forrest S, et al. Screening of healthcare workers for SARS-CoV-2 highlights the role of asymptomatic carriage in COVID-19 transmission. eLife. 2020 May 11;9:e58728.
79. Moynan D, Cagney M, Dhuthaigh AN, Foley M, Salter A, Reidy N, et al. The role of healthcare staff COVID-19 screening in infection prevention & control. J Infect. 2020 Sep;81(3):e53–4.
80. Treibel TA, Manisty C, Andiapen M, Pade C, Jensen M, Fontana M, et al. Asymptomatic health-care worker screening during the COVID-19 pandemic – Authors' reply. The Lancet. 2020 Oct;396(10260):1394–5.
81. Chow A, Htun HL, Kyaw WM, Lee LT, Ang B. Asymptomatic health-care worker screening during the COVID-19 pandemic. The Lancet. 2020 Oct;396(10260):1393–4.
82. Brown CS, Clare K, Chand M, Andrews J, Auckland C, Beshir S, et al. Snapshot PCR surveillance for SARS-CoV-2 in hospital staff in England. J Infect. 2020 Sep;81(3):427–34.

83. FDA Pfizer-BioNTech COVID-19 Vaccine [Internet]. [cited 2021 Jan 8]. Available from: https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/pfizer-biontech-covid-19-vaccine
84. Aldridge RW, Lewer D, Katikireddi SV, Mathur R, Pathak N, Burns R, et al. Black, Asian and Minority Ethnic groups in England are at increased risk of death from COVID-19: indirect standardisation of NHS mortality data. Wellcome Open Res. 2020;5:88.
85. Kumar N, Bhartiya S, Desai S, Mutha A, Beldar A, Singh T. Seroprevalence of Antibodies Against SARS-CoV-2 Among Health Care Workers in Mumbai, India. Asia Pac J Public Health. 2020 Nov 26;1010539520977307.
86. Voysey, Merryn and Costa Clemens, Sue Ann and Madhi. Single Dose Administration, And The Influence Of The Timing Of The Booster Dose On Immunogenicity and Efficacy Of ChAdOx1 nCoV-19 (AZD1222) Vaccine. Available from: https://ssrn.com/abstract=3777268
87. EUA Authorized Serology Test Performance. Available at: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-test-performance. Accessed 25th February 2021.
88. Dean AG, Sullivan KM, Soe MM. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version. www.OpenEpi.com, updated 2013/04/06, accessed 2021/03/21.
89. Algaissi A, Alfaleh MA, Hala S, Abujamel TS, Alamri SS, Almahboub SA, et al. SARS-CoV-2 S1 and N-based serological assays reveal rapid seroconversion and induction of specific antibody response in COVID-19 patients. Sci Rep. 2020 Dec;10(1):16561.
90. Pollán M, Pérez-Gómez B, Pastor-Barriuso R, Oteo J, Hernán MA, Pérez-Olmeda M, et al. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. The Lancet. 2020 Aug;396(10250):535–44.
91. Petersen LR, Sami S, Vuong N, Pathela P, Weiss D, Morgenthau BM, et al. Lack of Antibodies to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a Large Cohort of Previously Infected Persons. Clin Infect Dis. 2020 Nov 5;ciaa1685.
92. Kaufman HW, Chen Z, Meyer WA, Wohlgemuth JG. Insights from Patterns of SARS-CoV-2 Immunoglobulin G Serology Test Results from a National Clinical Laboratory, United States, March–July 2020. Popul Health Manag. 2021 Feb 1;24(S1):S-35-S-42.
93. Pelleau S, Woudenberg T, Rosado J, Donnadieu F, Garcia L, Obadia T, et al. Serological reconstruction of COVID-19 epidemics through analysis of antibody kinetics to SARS-CoV-2 proteins [Internet]. Infectious Diseases (except HIV/AIDS); 2021 Mar [cited 2021 Apr 29]. Available from: http://medrxiv.org/lookup/doi/10.1101/2021.03.04.21252532
94. Weidner L, Gänsdorfer S, Unterweger S, Weseslindtner L, Drexler C, Farcet M, et al. Quantification of SARS-CoV-2 antibodies with eight commercially available immunoassays. J Clin Virol. 2020 Aug;129:104540.
95. Harley K, Gunsolus IL. Comparison of the Clinical Performances of the Abbott Alinity IgG, Abbott Architect IgM, and Roche Elecsys Total SARS-CoV-2 Antibody Assays. Tang YW, editor. J Clin Microbiol. 2020 Dec 17;59(1):JCM.02104-20, e02104-20.
96. Kumar N, Bhartiya S, Singh T. Duration of anti-SARS-CoV-2 antibodies much shorter in India. Vaccine. 2021 Feb;39(6):886–8.
97. the UNICORN Consortium, Milani GP, Dioni L, Favero C, Cantone L, Macchi C, et al. Serological follow-up of SARS-CoV-2 asymptomatic subjects. Sci Rep. 2020 Dec;10(1):20048.
98. den Hartog G, Vos ERA, van den Hoogen LL, van Boven M, Schepp RM, Smits G, et al. Persistence of antibodies to SARS-CoV-2 in relation to symptoms in a nationwide prospective study. Clin Infect Dis. 2021 Feb 24;ciab172.
99. Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, et al. Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Infectious Diseases Group, editor.

Cochrane Database Syst Rev [Internet]. 2020 Jun 25 [cited 2021 Apr 9]; Available from: http://doi.wiley.com/10.1002/14651858.CD013652
100. Report of the profile of COVID-19 cases in healthcare workers in Ireland [Internet]. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/surveillance/covid-19casesinhealthcareworkers/COVID-19_HCW_weekly_report_13%2004%202021_v1.0%20website%20version.pdf
101. HPSC COVID-19 in Healthcare Workers [Internet]. HPSC COVID-19 in Healthcare Workers. 2021. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/surveillance/covid-19casesinhealthcareworkers/
102. Allen N. Prevalence of Antibodies to SARS-CoV-2 in Irish Healthcare Workers Phase 1 October 2020 [Internet]. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/research/precise/PRECISE%20Study%20Phase%201%20Interim%20Report%20January%202021.pdf
103. Health Information and Quality Authority; Advice to the National Public Health Emergency Team; duration of immunity following SARS-CoV-2 infection [Internet]. 2021. Available from: https://www.hiqa.ie/sites/default/files/2021-03/Duration-of-protective-immunity_Advice-to-NPHET-24%20-Feb-2021.pdf
104. Thompson MG, Burgess JL, Naleway AL, Tyner H, Yoon SK, Meece J, et al. Prevention and Attenuation of Covid-19 with the BNT162b2 and mRNA-1273 Vaccines. N Engl J Med. 2021 Jun 30;NEJMoa2107058.
105. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N Engl J Med. 2021 Feb 4;384(5):403–16.
106. Jara A, Undurraga EA, González C, Paredes F, Fontecilla T, Jara G, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. N Engl J Med. 2021 Jul 7;NEJMoa2107715.
107. Johnson & Johnson COVID-19 Vaccine Authorized by U.S. FDA For Emergency Use - First Single-Shot Vaccine in Fight Against Global Pandemic [Internet]. [cited 2021 Apr 15]. Available from: https://www.jnj.com/johnson-johnson-covid-19-vaccine-authorized-by-u-s-fda-for-emergency-usefirst-single-shot-vaccine-in-fight-against-global-pandemic
108. Lumley SF, O'Donnell D, Stoesser NE, Matthews PC, Howarth A, Hatch SB, et al. Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers. N Engl J Med. 2021 Feb 11;384(6):533–40.
109. Meyer B, Drosten C, Müller MA. Serological assays for emerging coronaviruses: Challenges and pitfalls. Virus Res. 2014 Dec;194:175–83.
110. Allen N, Ni Riain U, Conlon N, Ferenczi A, Carrion Martin AI, Domegan L, et al. Prevalence of Antibodies to SARS-CoV-2 in Irish Hospital Healthcare Workers. Epidemiol Infect. 2021 Apr 27;1–33.
111. COVID-19 cases in Ireland, daily update midnight 15/04/2021 [Internet]. Health Protection Surveillance Centre. 2021 [cited 2021 Apr 19]. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/casesinireland/epidemiologyofcovid-19inireland/COVID19%20Daily%20infographic.pdf
112. National Immunisation Advisory Committee (NIAC) Guidelines for Immunisation Chapter 5A-COVID-19 . [Internet]. 2021 [cited 2021 Jun 18]. Available from: https://www.hse.ie/eng/health/immunisation/hcpinfo/guidelines/
113. CDC Real-World Study Confirms Protective Benefits of mRNA COVID-19 Vaccines [Internet]. 2021 [cited 2021 Jun 16]. Available from: https://www.cdc.gov/media/releases/2021/p0329-COVID-19-Vaccines.html
114. Prevalence of Antibodies to SARS-CoV-2 in Irish Healthcare Workers Phase 1 October 2020 Interim Report [Internet]. 2021 [cited 2021 Jun 14]. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/research/precise/PRECISE%20Study%20Phase%201%20Report%20NPHET%2014th%20January%20.pdf

115. Poletti P, Tirani M, Cereda D, Guzzetta G, Trentini F, Marziano V, et al. Seroprevalence of and Risk Factors Associated With SARS-CoV-2 Infection in Health Care Workers During the Early COVID-19 Pandemic in Italy. JAMA Netw Open. 2021 Jul 6;4(7):e2115699.
116. Jacob JT, Baker JM, Fridkin SK, Lopman BA, Steinberg JP, Christenson RH, et al. Risk Factors Associated With SARS-CoV-2 Seropositivity Among US Health Care Personnel. JAMA Netw Open. 2021 Mar 10;4(3):e211283.
117. Ruhnke GW, Richterman A. Predictors of COVID-19 Seropositivity Among Healthcare Workers: An Important Piece of an Incomplete Puzzle. J Hosp Med [Internet]. 2021 May [cited 2021 Jun 15];16(5). Available from: https://www.journalofhospitalmedicine.com/jhospmed/article/239085/hospital-medicine/predictors-covid-19-seropositivity-among-healthcare
118. Madewell ZJ, Yang Y, Longini IM, Halloran ME, Dean NE. Household Transmission of SARS-CoV-2: A Systematic Review and Meta-analysis. JAMA Netw Open. 2020 Dec 14;3(12):e2031756.
119. Korth J, Wilde B, Dolff S, Frisch J, Jahn M, Krawczyk A, et al. SARS-CoV-2 Seroprevalence in Healthcare Workers in Germany: A Follow-Up Study. Int J Environ Res Public Health. 2021 Apr 25;18(9):4540.
120. Yamamoto S, Tanaka A, Oshiro Y, Ishii M, Ishiwari H, Konishi M, et al. Seroprevalence of SARS-CoV-2 antibodies in a national hospital and affiliated facility after the second epidemic wave of Japan. J Infect. 2021 May;S0163445321002590.
121. Kahlert CR, Persi R, Güsewell S, Egger T, Leal-Neto OB, Sumer J, et al. Non-occupational and occupational factors associated with specific SARS-CoV-2 antibodies among hospital workers – A multicentre cross-sectional study. Clin Microbiol Infect. 2021 May;S1198743X21002366.
122. Galanis P, Vraka I, Fragkou D, Bilali A, Kaitelidou D. Seroprevalence of SARS-CoV-2 antibodies and associated factors in healthcare workers: a systematic review and meta-analysis. J Hosp Infect. 2021 Feb;108:120–34.
123. Elfström KM, Blomqvist J, Nilsson P, Hober S, Pin E, Månberg A, et al. Differences in risk for SARS-CoV-2 infection among healthcare workers [Internet]. Infectious Diseases (except HIV/AIDS); 2021 Apr [cited 2021 Jun 15]. Available from: http://medrxiv.org/lookup/doi/10.1101/2021.03.30.21254653
124. Wicker S, Zielen S, Rose MA. Obstacles in the Motivation of Health Care Workers for Pertussis vaccination. Procedia Vaccinol. 2010;2(1):106–8.
125. Gao Z, Xu Y, Sun C, Wang X, Guo Y, Qiu S, et al. A systematic review of asymptomatic infections with COVID-19. J Microbiol Immunol Infect. 2021 Feb;54(1):12–6.
126. Long QX, Tang XJ, Shi QL, Li Q, Deng HJ, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med. 2020 Aug;26(8):1200–4.
127. Hawkins RB, Charles EJ, Mehaffey JH. Socio-economic status and COVID-19–related cases and fatalities. Public Health. 2020 Dec;189:129–34.
128. Truong N, Asare AO. Assessing the effect of socio-economic features of low-income communities and COVID-19 related cases: An empirical study of New York City. Glob Public Health. 2021 Jan 2;16(1):1–16.
129. Valdes AM, Moon JC, Vijay A, Chaturvedi N, Norrish A, Ikram A, et al. Longitudinal assessment of symptoms and risk of SARS-CoV-2 infection in healthcare workers across 5 hospitals to understand ethnic differences in infection risk. EClinicalMedicine. 2021 Apr;34:100835.
130. Martyn EM, Whitaker H, Gil E, Ighomereho P, Lambe G, Conley R, et al. Disproportionate impact of SARS-CoV-2 on ethnic minority and frontline healthcare workers: A cross-sectional seroprevalence survey at a North London hospital. J Infect. 2021 Jun;82(6):276–316.
131. Eyre DW, Lumley SF, Wei J, Cox S, James T, Justice A, et al. Quantitative SARS-CoV-2 anti-spike responses to Pfizer-BioNTech and Oxford-AstraZeneca vaccines by previous infection status. Clin Microbiol Infect. 2021 Jun;S1198743X21002895.
132. CDC Morbidity and Mortality Weekly Report (MMWR) - Interim Estimates of Vaccine Effectiveness of BNT162b2 and mRNA-1273 COVID-19 Vaccines in Preventing SARS-CoV-2 Infection Among Health Care

Personnel, First Responders, and Other Essential and Frontline Workers — Eight U.S. Locations, December 2020–March 2021 [Internet]. 2021 [cited 2021 Jun 16]. Available from: https://www.cdc.gov/mmwr/volumes/70/wr/mm7013e3.htm
133. CDC COVID-19 Vaccine Breakthrough Case Investigations Team, CDC COVID-19 Vaccine Breakthrough Case Investigations Team, Birhane M, Bressler S, Chang G, Clark T, et al. COVID-19 Vaccine Breakthrough Infections Reported to CDC — United States, January 1–April 30, 2021. MMWR Morb Mortal Wkly Rep. 2021 May 28;70(21):792–3.
134. Torjesen I. Covid-19: One in four vaccinated people living in households with a covid-19 case become infected, study finds. BMJ. 2021 Oct 29;n2638.
135. Pritchard E, Matthews PC, Stoesser N, Eyre DW, Gethings O, Vihta KD, et al. Impact of vaccination on new SARS-CoV-2 infections in the United Kingdom. Nat Med [Internet]. 2021 Jun 9 [cited 2021 Jun 16]; Available from: http://www.nature.com/articles/s41591-021-01410-w
136. Shamier MC, Tostmann A, Bogers S, de Wilde J, IJpelaar J, van der Kleij WA, et al. Virological characteristics of SARS-CoV-2 vaccine breakthrough infections in health care workers [Internet]. Infectious Diseases (except HIV/AIDS); 2021 Aug [cited 2021 Nov 15]. Available from: http://medrxiv.org/lookup/doi/10.1101/2021.08.20.21262158
137. Eyre DW, Taylor D, Purver M, Chapman D, Fowler T, Pouwels KB, et al. Effect of Covid-19 Vaccination on Transmission of Alpha and Delta Variants. N Engl J Med. 2022 Feb 24;386(8):744–56.
138. Whitaker HJ, Elgohari S, Rowe C, Otter AD, Brooks T, Linley E, et al. Impact of COVID-19 vaccination program on seroprevalence in blood donors in England, 2021. J Infect. 2021 May;S0163445321002243.
139. Amjadi MF, O'Connell SE, Armbrust T, Mergaert AM, Narpala SR, Halfmann PJ, et al. Specific COVID-19 Symptoms Correlate with High Antibody Levels against SARS-CoV-2. ImmunoHorizons. 2021 Jun 17;5(6):466–76.
140. Allen N, Brady M, Carrion Martin AI. SARS-CoV-2 Antibody Testing in Health Care Workers: A Comparison of the Clinical Performance of Three Commercially Available Antibody Assays. Microbiol Spectr.
141. Interim analysis of COVID-19 vaccine effectiveness against Severe Acute Respiratory Infection due to laboratory-confirmed SARS-CoV-2 among individuals aged 30 years and older, ECDC multi-country study – second update [Internet]. 2022. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/covid-19-vaccine-effectiveness-interim-analysis-second-update_0.pdf
142. Vaccine effectiveness, Burden and Impact Studies (VEBIS) of COVID-19 and Influenza [Internet]. epiconcept.fr. 2021 [cited 2022 Dec 13]. Available from: https://www.epiconcept.fr/en/epidemio-project/vaccine-effectiveness-burden-and-impact-studies-vebis-of-covid-19-and-influenza/

Appendix 1 – Supplementary tables and figures

Table A. Response rate by HCW type, PRECISE 1, October 2020

HCW role	St James's Hospital Response Rate			University Hospital Galway Response Rate			Combined Response Rate		
	N	n	%	N	n	%	N	n	%
Admin	693	454	66%	639	349	55%	1,332	803	60%
Medical/dental	621	460	74%	814	522	64%	1,435	982	68%
Nursing/ midwifery	1,802	1,045	58%	1,689	1,019	60%	3,491	2,064	59%
Allied health	742	616	83%	648	475	73%	1,390	1,091	78%
General support	412	255	62%	240	179	75%	652	434	67%
Health care assistant	422	157	37%	316	129	41%	738	286	39%
Other	-	55		-	72		-	127	
Total	4,692	3042	65%	4,346	2,745	63%	9,038	5,788	64%

Table B. Comparison of all staff and study participants in St James's Hospital by healthcare worker role, PRECISE 1, October 2020

HCW role	St James's Hospital all staff		St James's Hospital study participants		Difference	
	N	%	N	%	N	%
Admin	693	15	454	15	239	0.1
Medical/dental	621	13	460	15	161	1.9
Nursing/ midwifery	1,802	38	1,045	35	757	-3.8
Allied health	742	16	616	20	126	4.5
General support	412	8.8	255	8.4	157	-0.4
Health care assistant	422	9.0	157	5.2	265	-3.8
Other	-	-	55	1.8	-	-
Total	4,692	100	3042	100	1650	-

Table C. Comparison of all staff and study participant in University Hospital Galway by healthcare worker role, PRECISE 1, October 2020

HCW role	University Hospital Galway all staff		University Hospital Galway study participants		Difference	
	N	%	N	%	N	%
Admin	639	15	349	13	290	-2
Medical/dental	814	19	522	19	291	0.4
Nursing/ midwifery	1,689	39	1,019	37	670	-1.8
Allied health	648	15	475	17	173	2.4
General support	240	5.5	179	6.5	61	1
Health care assistant	316	7.3	129	4.7	187	-2.6
Other	-	-	72	2.6	-	-
Total	4,346	100	2,745	100	1600	-

Table D. Comparison of all staff and study participants by healthcare worker role, PRECISE 1, October 2020

HCW role	All invited staff		All study participants		Difference	
	N	%	N	%	N	%
Admin	1,332	15	803	13	529	-1.3
Medical/dental	1,435	16	982	17	452	1.1
Nursing/ midwifery	3,491	39	2,064	36	1427	-2.9
Allied health	1,390	15	1,091	19	299	3.2
General support	652	7.2	434	7.5	218	0.3
Health care assistant	738	8.2	286	4.9	452	-3.3
Other	-	-	127	2.19	-	-
Total	9,038	100	5,788	100	3250	

Table E. SARS-CoV-2 Seroprevalence by healthcare worker role in SJH, PRECISE 1, October 2020

HCW role	Participated SJH		Positive Results SJH			P-value*
	N	% of group	N	% of tested	95% Confidence Intervals (%)	
Admin	454	66	44	9.7	7.1-13	<.001
Medical/dental	460	74	66	14	11-18	
Nursing/ midwifery	1,045	58	215	21	18-23	
Allied health	616	83	61	9.9	7.7-13	
General support	255	62	30	12	8.1-16	
Health care assistant	157	37	42	27	20-34	
Other	55		6	11	4.1-22	
Total	3042	65	466	15	14-16	

*Calculated using the Chi-Square test

Table F. SARS-CoV-2 Seroprevalence by healthcare worker role in UHG, PRECISE 1, October 2020

HCW role	Participated UHG		Positive Results UHG			P-value*
	N	% of group	N	% of tested	95% Confidence Intervals (%)	
Admin	349	55	4	1.2	0.3-2.9	<.001
Medical/dental	522	64	36	6.9	4.9-9.4	
Nursing/ midwifery	1,019	60	48	4.7	3.5-6.2	
Allied health	475	73	12	2.5	1.3-4.4	
General support	179	75	3	1.7	0.4-4.8	
Health care assistant	129	41	8	6.2	2.7-12	
Other	72		1	1.4	0.1-7.5	
Total	2,745	63	112	4.1	3.4-4.9	

* Calculated using the Chi-Square test

Table G. Seroprevalence by healthcare worker role, both hospitals, PRECISE 1, October 2020

HCW role	Participated		Positive Results			P-value*
	N	% of group	N	% of tested	95% Confidence Intervals (%)	
Admin	803	60	48	6.0	4.4-7.9	
Medical/dental	982	68	102	10	8.5-13	
Nursing/ midwifery	2,064	59	263	13	11-14	
Allied health	1,091	78	73	6.7	5.3-8.4	
General support	434	67	33	7.6	5.3-11	<.001
Health care assistant	286	39	50	18	13-22	
Other	127	-	7	5.5	2.2-11	
Total	5,788	64	576	10	9.2-11	

* Calculated using the Chi-Square test

Characteristics of HCW with SARS-CoV-2 antibodies detected

The HCW with detectable SARS-CoV-2 antibodies had a median age of 36.7 (IQR 28.6-46.9) and were 73% female, 67% Irish and 21.4% BAME. 46% were nurses, and 89% had at least third level education. Of them, 95% were living with other people, and 41% were living with other HCW. 16% had not experienced symptoms consistent with COVID-19 at any stage. 82% had previously had at least one PCR test for COVID-19, and 61% had previously had a positive test. (Table H).

Table H. Characteristics of the HCW with SARS-CoV-2 antibodies detected (n=576), by hospital, PRECISE 1, October 2020

Participant characteristics		St James's Hospital (n=464)		University Hospital Galway (n=112)		All with SARS-CoV-2 IgG antibodies detected (n=576)	
Age	Median age (IQR)	37	29-48	36	29-42	37	29-47
		N	**%**	**N**	**%**	**N**	**%**
Age groups	18-29	148	32	29	26	177	31
	30-39	121	26	47	42	168	29
	40-49	99	21	25	22	124	32
	50-59	70	15	7	6.3	77	13
	Over 60	26	5.6	4	3.6	30	5.2
Sex	Female	347	75	75	67	422	73
	Male	117	25	37	33	154	27

Ethnicity	Irish	305	66	80	71	385	67
	Any other white background	44	9.5	18	16	62	11
	Any Asian background	94	20	13	12	107	19
	African or other black background	15	3.2	1	0.9	16	2.8
	Other	6	1.3	-	-	6	1.0
Country of birth*	Ireland	291	63	82	73	373	65
	United Kingdom	25	5.4	7	6.3	32	5.6
	India	46	9.9	8	7.1	54	9.4
	Philippines	45	9.7	2	1.8	47	8.2
	Poland	7	1.5	3	2.7	10	1.7
	USA	3	0.7	-	-	3	0.5
	Other	47	10	10	8.9	57	9.9
Education	Primary	4	0.9	-	-	4	0.7
	Secondary	53	11	8	7.1	61	11
	Third level	229	49	54	48	283	49
	Post-graduate	178	38	50	45	228	40
Role	Admin	44	9.5	4	3.6	48	8.3
	Medical/dental	66	14	36	32	102	18
	Nursing/ midwifery	215	46	48	43	263	46
	Allied health	61	13	12	11	73	13
	General support	30	6.5	3	2.7	33	5.7
	Health care assistant	42	9.1	8	7.1	50	8.7
	Other	6	1.3	1	0.9	7	1.2
Lives with	Alone	21	4.5	7	6.3	28	4.9
	With others	441	95.0	105	93.8	546	94.8
	Missing	2	0.4	-	-	2	0.3
Lives with HCW	Yes	193	42	40	37	234	41
	No	262	57	70	63	332	58
	Missing	9	1.9	1	0.9	10	1.7
Previous COVID-19 like symptoms	No symptoms	70	15	20	18	90	16
	Had symptoms	392	85	92	82	484	84
	Missing	2	0.4	-	-	2	0.4
Previous COVID-19 PCR test	Yes	385	83	89	80	474	82
	No	79	17	23	21	102	18
Previous positive COVID-19 PCR test	Yes	277	60	73	65	350	61
	No	187	40	39	35	226	39

Table 2c. Prevalence of SARS-CoV-2 IgG antibodies by participant characteristics, St James's Hospital, PRECISE 1, October 2020

Participant characteristics		Total	SARS-CoV-2 IgG detected		P-value*
		N	n	% (95% CI)	
Age groups	18-29	728	148	20 (17 - 23)	<0.001
	30-39	831	121	15 (12 -17)	
	40-49	793	99	13 (10 -15)	
	50-59	532	70	13 (10 - 16)	
	Over 60	158	26	17 (11 - 23)	
Sex	Female	2,326	347	15 (13 -16)	0.355
	Male	716	117	16 (14 -19)	
Ethnicity	Irish	2,262	304	13 (12 - 15)	<0.001
	Any other white background	267	44	17 (12 - 22)	
	African and any other black background	65	15	23 (14 - 35)	
	Asian background	393	94	24 (20 - 29)	
	Other	55	7	13 (5.3 - 25)	
Country of birth*	Ireland	2,182	291	13 (12 - 15)	<0.001
	United Kingdom	152	25	16 (11 - 23)	
	India	201	46	23 (17 -29)	
	Philippines	166	45	27 (21 - 35)	
	Poland	24	7	29 (13 -51)	
	USA	22	3	14 (2.9 - 35)	
	Other	295	47	16 (12 - 20)	
Education	Primary	27	4	15 (4 - 34)	0.017
	Secondary	420	53	13 (10 -16)	
	Third level	1,300	229	18 (16 -20)	
	Post-graduate	1,295	178	14 (12 -16)	
Role	Admin	454	44	10 (7.1 - 13)	<0.001
	Medical/dental	460	66	14 (11 - 18)	
	Nursing/ midwifery	1,045	215	21 (18 -23)	
	Allied health	616	61	10 (7.7 - 13)	
	General support	255	30	12 (8.1 -16)	
	Health care assistant	157	42	27 (20 - 34)	
	Other	55	6	11 (4.1 - 22)	
Lives with	Alone	256	21	8 (5.2 -12)	0.004
	With others	2,768	441	16 (15 -17)	
	Missing	18	2	11 (1.4 -35)	
Lives with HCW	Yes	928	193	21 (18 -24)	<0.001
	No	2,060	262	13 (11 - 14)	
	Missing	54	9	17 (7.9 – 29)	

* Calculated using the Chi-Square test

Table 2d. Prevalence of SARS-CoV-2 IgG antibodies by COVID-19 related characteristics, St James's Hospital, PRECISE 1, October 2020

Participant characteristics		Total	SARS-CoV-2 IgG detected		P-value*
		N	n	% (95% CI)	
Contact of a COVID-19 case	Yes	1,185	272	23 (21 - 25)	<0.001
	No	1,847	190	10 (8.9 - 12)	
	Missing	10	2	20 (2.5 - 56)	
Setting of close contact	Contact at work	1,039	225	22 (19 -24)	<0.001
	Contact outside of work	146	47	32 (25 - 40)	
Workplace exposure	Daily contact with COVID-19 patients	510	108	21 (18 - 25)	<0.001
	Daily contact with patients without COVID	1,611	269	17 (15 -19)	
	No patients contact	918	87	9.5 (7.7 - 12)	
Previous COVID-19 like symptoms	No symptoms	1,359	72	5.3 (4.2 – 6.6)	<0.001
	Had symptoms	1,683	392	23 (21 - 25)	
Previous COVID-19 PCR test	Yes	1,685	385	23 (21 - 25)	<0.001
	No	1,353	79	5.8 (4.6 - 7.2)	
Previous positive COVID-19 PCR test	Yes	292	277	94.9 (91.7 - 97.1)	<0.001
	No	2,746	187	6.8 (5.8 – 7.8)	

* Calculated using the Chi-Square test

Table 2e. Prevalence of SARS-CoV-2 IgG antibodies by participant characteristics, University Galway Hospital, PRECISE 1, October 2020

Participant characteristics		Total	SARS-CoV-2 IgG detected		P-value*
		N	n	% (95% CI)	
Age groups	18-29	622	29	4.7 (3.1 – 6.6)	0.002
	30-39	786	47	6.0 (4.4 – 7.9)	
	40-49	722	25	3.5 (2.2 – 5.1)	
	50-59	469	7	1.5 (0.6 – 3.0)	
	Over 60	146	4	2.7 (0.8 – 6.9)	
Sex	Female	2,152	75	3.5 (2.8 – 4.3)	0.003
	Male	592	37	6.3 (4.4 – 8.5)	
Ethnicity	Irish	2,182	80	3.7 (2.9 – 4.5)	0.031
	Any other white background	284	18	6.3 (3.8 - 9.8)	
	African and any other black background	48	1	2.1 (0.1 - 11)	
	Asian background	184	13	7.1 (3.8 - 12)	
	Other	46	0	-	
Country of birth*	Ireland	2,091	82	3.9 (3.1 – 4.8)	0.229
	United Kingdom	192	7	3.7 (1.5 - 7.4)	
	India	98	8	8.2 (3.6 - 15)	
	Philippines	25	2	8.0 (1.0 - 26)	
	Poland	48	3	6.3 (0.1 - 17)	
	USA	38	0	-	
	Other	253	10	4.0 (1.9 – 7.2)	
Education	Primary	2	0	-	0.690
	Secondary	264	8	3.0 (1.3 – 5.9)	
	Third level	1,245	54	4.3 (3.3 – 5.6)	
	Post-graduate	1,232	50	4.1 (3.0 – 5.3)	
Role	Admin	349	4	1.2 (3.1 – 2.9)	<0.001
	Medical/dental	522	36	6.9 (4.9 – 9.4)	
	Nursing/ midwifery	1,019	48	4.7 (3.5 - 6.2)	
	Allied health	475	12	2.5 (1.3 – 4.4)	
	General support	179	3	1.7 (0.3 – 4.8)	
	Health care assistant	129	8	6.2 (2.7 - 12)	
	Other	72	1	1.4 (0.1 -7.5)	
Lives with	Alone	223	7	3.1 (1.3 – 6.4)	0.658
	With others	2,518	105	4.2 (3.4 – 5.0)	
	Missing	4	0	-	
Lives with HCW	Yes	839	41	4.9 (3.5 – 6.6)	0.354
	No	1,859	70	3.8 (3.0 – 4.7)	
	Missing	47	1	2.1 (0.1 – 11.3)	

* Calculated using the Chi-Square test or the Fisher exact test

Table 2f. Prevalence of SARS-CoV-2 IgG antibodies by COVID-19 related characteristics, University Galway Hospital, PRECISE 1, October 2020

Participant characteristics		Total N	SARS-CoV-2 IgG detected n	SARS-CoV-2 IgG detected % (95% CI)	P-value*
Contact of a COVID-19 case	Yes	519	53	10 (7.7 – 13)	<0.001
	No	2,224	59	2.7 (2.0 – 3.4)	
	Missing	2	0	-	
Setting of close contact	Contact at work	456	44	9.7 (7.0 – 12.7)	0.255
	Contact outside of work	63	9	14 (6.7 – 25.4)	
Workplace exposure	Daily contact with COVID-19 patients	392	28	7.1 (5.0 – 10)	<0.001
	Daily contact with patients without COVID	1,634	75	4.6 (3.7 -5.7)	
	No patients contact	717	9	1.3 (0.1 – 2.4)	
Previous COVID-19 like symptoms	No symptoms	1,517	20	1.3 (0.8 – 2.0)	<0.001
	Had symptoms	1,228	92	7.5 (6.1 – 9.1)	
Previous COVID-19 PCR test	Yes	1,093	89	8.1 (6.6 – 9.9)	<0.001
	No	1,650	23	1.4 (0.9 – 2.1)	
Previous positive COVID-19 PCR test	Yes	75	73	97.3 (90.7 – 99.7)	<0.001
	No	2,668	39	1.5 (1.0 – 2.0)	

* Calculated using the Chi-Square test or the Fisher exact test

Table 3a. Association between risk factors and the presence of SARS-CoV-2 IgG antibodies, Saint James's Hospital, PRECISE 1, October 2020

Participant characteristics		Unadjusted relative risk (95% CI)	P-value	Adjusted relative risk (95% CI)	P-value
Age groups	18-29	1.5 (1.2 – 2.0)	0.001	1.3 (1.0 – 1.8)	0.029
	30-39	1.1 (0.8 - 1.5)	0.468	1.0 (0.7 – 1.3)	0.886
	40-49	0.9 (0.7 – 1.3)	0.718	0.9 (0.7 – 1.2)	0.407
	50-59	Ref.			
	Over 60	1.3 (0.8 -1.9)	0.289	1.3 (0.9 – 1.9)	0.214
Sex	Female	Ref.			
	Male	1.1 (0.9 – 1.3)	0.353	1.1 (0.9 – 1.4)	0.318
Ethnicity	Irish	Ref.			
	Any other white background	1.2 (0.9 – 1.6)	0.168	1.2 (0.9 - 1.5)	0.309
	African and other black background	1.7 (1.1 – 2.7)	0.020	1.4 (0.9 – 2.2)	0.137
	Asian background	1.8 (1.4 – 2.2)	<0.001	1.3 (1.0 – 1.6)	0.052
	Other	0.9 (0.5 -1.9)	0.879	0.7 (0.3 – 1.5)	0.395
Country of birth	Ireland	Ref.			Did not enter
	India	1.7 (1.3 – 2.3)	<0.001		
	Philippines	2.0 (1.6 – 2.7)	<0.001		
	United Kingdom	1.2 (0.8 – 1.8)	0.272		
	Poland	2.2 (1.2 – 4.1)	0.015		
	USA	1.0 (0.4 - 2.9)	0.967		
	Other	1.2 (0.9 – 1. 6)	0.218		
Education	Primary	1.1 (0.4 – 2.7)	0.872		Did not enter
	Secondary	0.9 (0.7 – 1.2)	0.558		
	Third level	1.3 (1.1 - 1.5)	0.007		
	Post-graduate	Ref.			
Role	Admin	Ref.			
	Doctor\Dental	1.5 (1.0 - 2.2)	0.032	1.0 (0.7 – 1.5)	0.840
	Nursing	2.1 (1.6 – 2.9)	<0.001	1.6 (1.1 – 2.2)	0.013
	HCA	2.8 (1.9– 4.0)	<0.001	2.0 (1.3 – 3.0)	0.002
	General support	1.2 (0.8 – 1.9)	0.386	1.0 (0.6 – 1.5)	0.870
	Allied HCW	1.0 (0.7 – 1.5)	0.909	0.9 (0.6 – 1.3)	0.522
	Other	1.1 (0.5 – 2.8)	0.773	1.2 (0.5 – 2.6)	0.734

Lives with	Alone	Ref.			
	With others	1.9 (1.3 – 3.0)	0.002	1.6 (1.0 – 2.4)	0.037
Lives with HCW	No	Ref.			
	Yes	1.6 (1.4 - 1.9)	<0.001	1.6 (1.0 – 2.4)	0.037
Contact of a COVID-19 case	No	Ref.			Did not enter
	Yes	2.2 (1.9 – 2.6)	<0.001		
Close contact at work **	No	1.5 (1.1 – 1.9)	0.003		Did not enter
	Yes	Ref.			
Workplace exposure to COVID-19 patients	No patients contact	Ref.			
	Daily contact with patients without COVID	1.8 (1.4 – 2.2)	<0.001	1.3 (1.0 – 1.7)	0.0398
	Daily contact with COVID-19 patients	2.2 (1.7 – 2.9)	<0.001	1.4 (1.0 – 1.9)	0.036
Previous COVID-19 like symptoms	No	Ref.			Did not enter
	Yes	4.4 (3.5 – 5.6)	<0.001		

**Calculated for close contacts of COVID-19 cases only (n=1,185)

Table 3b. Association between risk factors and the presence of SARS-CoV-2 IgG antibodies, University Hospital Galway, PRECISE 1, October 2020

Participant characteristics		Unadjusted relative risk (95% CI)	P-value	Adjusted relative risk (95% CI)	P-value
Age groups	18-29	3.2 (1.4 – 7.1)	0.006	2.7 (1.2 – 6.2)	0.018
	30-39	4.0 (1.8 – 8.8)	<0.001	3.5 (1.6 – 7.6)	0.002
	40-49	2.3 (1.0 – 5.3)	0.047	2.0 (1.0 – 5.0)	0.064
	50-59	Ref.			
	Over 60	1.8 (0.5 -6.2)	0.327	2.1 (0.6 – 6.9)	0.241
Sex	Female	Ref.			
	Male	1.8 (1.2 – 2.6)	0.003	1.9 (1.2 – 3.0)	0.005
Ethnicity	Irish	Ref.			
	Any other white background	1.7 (1.0 – 2.8)	0.031	1.6 (1.0 - 2.6)	0.072
	African and other black background	0.6 (0.1 – 4.0)	0.570	0.4 (0.1 – 2.5)	0.295
	Asian background	1.9 (1.1 – 3.4)	0.023	1.0 (0.6 – 1.9)	0.921
	Other	no observation	-	-	-
Country of birth	Ireland	Ref.			Did not enter
	India	2.1 (1.0 – 4.2)	0.039		
	Philippines	2.0 (0.5 – 7.8)	0.29		
	United Kingdom	0.9 (0.4 – 2.0)	0.850		
	Poland	1.6 (0.5 – 4.9)	0.413		
	USA	no observation	-		
	Other	1.0 (0.5 – 1.9)	0.981		
Education	Primary	no observation	-		Did not enter
	Secondary	0.7 (0.4 – 1.6)	0.436		
	Third level	1.1 (0.7 - 1.6)	0.729		
	Post-graduate	Ref.			
Role	Admin	Ref.			
	Doctor\Dental	6.0 (2.2 - 17)	0.001	2.4 (0.8 – 7.6)	0.126
	Nursing	4.1 (1.5 – 11)	0.006	2.2 (0.7 – 6.7)	0.157
	HCA	5.4 (1.7 – 14)	0.005	2.7 (0.8 – 9.4)	0.122
	General support	1.5 (0.3 - 6.5)	0.616	0.6 (0.1 – 2.7)	0.445
	Allied HCW	2.2 (0.7 – 6.8)	0.168	1.4 (0.4 – 4.4)	0.571
	Other	1.2 (0.1 – 11)	0.863	0.6 (0.1 – 5.0)	0.607

Lives with	Alone	Ref.			
	With others	1.3 (0.6 – 2.8)	0.460	1.0 (0.5 – 2.2)	0.920
Lives with HCW	No	Ref.			
	Yes	1.3 (0.9 -1.9)	0.175	0.9 (0.6 – 1.4)	0.736
Contact of a COVID-19 case	No	Ref.			Did not enter
	Yes	3.9 (2.7 – 5.5)	<0.001		
Close contact at work **	No	1.5 (0.8 – 2.9)	0.249		Did not enter
	Yes	Ref.			
Workplace exposure to COVID-19 patients	No patients contact	Ref.			
	Daily contact with patients without COVID	3.7 (1.8 – 7.3)	<0.001	2.0 (0.9 – 4.4)	0.069
	Daily contact with COVID-19 patients	5.7 (2.7 –12)	<0.001	3.1 (1.3 – 6.9)	0.009
Previous COVID-19 like symptoms	No	Ref.			Did not enter
	Yes	5.7 (3.5– 9.2)	<0.001		

**Calculated for close contacts of COVID-19 cases only (n=519)

Appendix 4A Characteristics of participants tested, by serology assay**,** PRECISE 1, October 2020

		Abbott SARS-CoV-2 IgG		**Roche Anti-SARS-CoV-2**		**Wantai SARS-CoV-2 Antibody ELISA**		**Total participants**	
Total participants tested		(n=5,788)		(n=5,787)		(n=485)		(n=5,788)	
		n	%	n	%	n	%	n	%
Age group (years)	18-29	1350	23.3	1350	23.3	139	28.7	1350	23.3
	30-39	1617	27.9	1617	27.9	139	28.7	1617	27.9
	40-49	1516	26.2	1515	26.2	111	22.9	1516	26.2
	50-59	1001	17.3	1001	17.3	68	14.0	1001	17.3
	60+	304	5.3	304	5.3	28	5.8	304	5.3
Sex	Female	4478	77.4	4478	77.4	349	72.0	4478	77.4
	Male	1309	22.6	1308	22.6	136	28.0	1309	22.6
	Unknown	1	0.0	1	0.0	0	0.0	1	0.0
Ethnicity	Irish	4444	76.8	4444	76.8	313	64.5	4444	76.8
	Any other white background	552	9.5	551	9.5	54	11.1	552	9.5
	Any Asian background	577	10.0	577	10.0	101	20.8	577	10.0
	Any African or black background	113	2.0	113	2.0	13	2.7	113	2.0
	Other	101	1.7	101	1.7	4	0.8	101	1.7
	Unknown	1	0.0	1	0.0	0	0	1	0.0
Role	Nursing/midwifery	2064	35.7	2064	35.7	230	47.4	2064	35.7
	Allied health	1091	18.8	1091	18.9	57	11.8	1091	18.8
	Medical/dental	983	17.0	982	17.0	93	19.2	983	17.0
	Admin	803	13.9	803	13.9	33	6.8	803	13.9
	General support	434	7.5	434	7.5	21	4.3	434	7.5
	Health care assistant	286	4.9	286	4.9	45	9.3	286	4.9
	Other	127	2.2	127	2.2	6	1.2	127	2.2

Appendix 4B COVID-19 related characteristics of participants tested, by serology assay**,** PRECISE 1, October 2020

		Abbott SARS-CoV-2 IgG		Roche Anti-SARS-CoV-2		Wantai SARS-CoV-2 Antibody ELISA		Total participants	
Total participants tested		(n = 5,788)		(n = 5,787)		(n = 485)		(n = 5,788)	
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Close contact with a case of	Yes	1705	29.5	1704	29.4	258	53.2	1705	29.5
	No	4071	70.3	4071	70.3	225	46.4	4071	70.3
	Unknown	12	0.2	12	0.2	2	0.4	12	0.2
Main type of patient contact*	Daily contact with known/suspected COVID-19	903	15.6	902	15.6	109	22.5	903	15.6
	Daily contact with patients without COVID-19	3245	56.1	3245	56.1	299	61.6	3245	56.1
	No patient contact	1635	28.2	1635	28.3	77	15.9	1635	28.2
	Unknown	5	0.1	5	0.1	0	0.0	5	0.1
Previous COVID-19 symptoms	No symptoms	2869	49.6	2868	49.6	102	21.0	2869	49.6
	Had symptoms	2911	50.3	2911	50.3	381	78.6	2911	50.3
	Unknown	8	0.1	8	0.1	2	0.4	8	0.1
Severity of symptoms	Minor symptoms	2159	74.2	2159	74.2	193	50.7	2159	74.2
	Significant symptoms	701	24.1	701	24.1	161	42.3	701	24.1
	Severe symptoms (hospitalised)	51	1.8	51	1.8	27	7.1	51	1.8
Previous COVID-19 PCR test	Yes	2779	48.0	2778	48.0	380	78.4	2779	48.0
	No	3003	51.9	3003	51.9	105	21.6	3003	51.9
	Unknown	6	0.1	6	0.1	0	0.0	6	0.1
Previous positive COVID-19	Yes	367	6.3	367	6.3	259	53.4	367	6.3
	No	5415	93.6	5414	93.6	226	46.6	5415	93.6
	Unknown	6	0.1	6	0.1	0	0.0	6	0.1

*Participants were asked which one describes MOST of their current work

Appendix 4C Characteristics of participants who had a previously PCR-confirmed infection and were negative by serology testing (n=17), PRECISE 1, October 2020

		N	%
Total		17	
Age group	18-29	5	29.4
	30-39	4	23.5
	40-49	4	23.5
	50-59	4	23.5
	60+	0	0.0
Sex	Female	14	82.4
	Male	3	17.6
Ethnicity	Irish	16	94.1
	Any other white background	1	5.9
Role	Nursing/midwifery	7	41.2
	Medical/dental	4	23.5
	Admin	1	5.9
	Allied health	2	11.8
	Healthcare assistant	2	11.8
	General support	1	5.9
Close contact with a case of COVID-19	Yes	7	41.2
	No	10	58.8
Main type of contact with COVID-19 patients	Daily contact with	3	17.6
	Daily contact with patients without	11	64.7
	No patient contact	3	17.6
Previous COVID-19 symptoms	No symptoms	2	11.8
	Had symptoms	15	88.2
Severity of symptoms	Minor symptoms	7	46.7
	Significant symptoms	8	53.3
	Severe symptoms (hospitalised)	0	0.0
Number of months since positive PCR test	Less than one month	1	5.9
	Five months	1	5.9
	Six months	15	88.2

Appendix 4D Characteristics of participants who had a previously PCR-confirmed infection and their test results on Abbott SARS-CoV-2 IgG (n=367)**,** PRECISE 1, October 2020

			All	**Grayzone**		**Positiv**		**Negati**	
Total (n)		367		93		15		12	
		n	%	n	%	n	%	n	%
Age group	18-29	111	30.2	32	34	38	25	41	33
	30-39	108	29.4	29	31	36	24	43	34
	40-49	79	21.5	20	21	42	28	17	13
	50-59	50	13.6	5	5.	26	17	19	15
	60+	19	5.2	7	7.	8	5.	4	3.
Sex	Female	269	73.3	68	73	99	66	10	82
	Male	98	26.7	25	26	51	34	22	17
Ethnicity	Irish	237	64.6	64	68	79	52	94	75
	Any other white background	44	12.0	9	9.	23	15	12	9.
	Any Asian background	72	19.6	16	17	42	28	14	11
	Any African or black background	10	2.7	3	3.	5	3.	2	1.
	Other	4	1.1	1	1.	1	0.	2	1.
Role	Nursing/midwifery	181	49.3	57	61	69	46	55	44
	Allied health	42	11.4	8	8.	15	10	19	15
	Medical/dental	73	19.9	11	11	33	22	29	23
	Administration	24	6.5	6	6.	9	6.	9	7.
	General support	12	3.3	2	2.	5	3.	5	4.
	Health care assistant	32	8.7	8	8.	19	12	5	4.
	Other	3	0.8	1	1.	0	0.	2	1.

Appendix 4E COVID-19 related characteristics of participants who had a previously PCR-confirmed infection and their test results on Abbott SARS-CoV-2 IgG (n=367)**,** PRECISE 1, October 2020

		All		**Grayzon**		**Positive**		**Negativ**	
Total (n)		367		93		150		124	
		n	%	n	%	n	%	n	%
Close contact with a case of	Yes	21	57.	56	60.	90	60.	65	52.
	No	15	42.	37	39.	58	38.	59	47.
	Unknown	2	0.5	0	0.0	2	1.3	0	0.0
Main type of patient contact*	Daily contact with known/suspected	88	24.	22	23.	40	26.	26	21.
	Daily contact with patients without	21	59.	56	60.	88	58.	74	59.
	No patient contact	61	16.	15	16.	22	14.	24	19.
Symptoms at time of previous	No symptoms	7	1.9	1	1.1	3	2.0	3	2.4
	Had symptoms	35	97.	92	98.	145	96.	12	97.
	Unknown	2	0.5	0	0.0	2	1.3	0	0.0
Severity of symptoms	Minor symptoms	15	41.	38	43.	51	35.	62	51.
	Significant symptoms	17	48.	48	54.	75	51.	55	45.
	Severe symptoms (hospitalised)	29	7.9	6	6.8	19	13.	4	3.3
Number of months since positive PCR test	Less than one month	8	2.2	1	1.1	6	4.0	1	0.8
	One month	8	2.2	0	0.0	8	5.3	0	0.0
	Two months	4	1.1	0	0.0	4	2.7	0	0.0
	Three months	1	0.3	0	0.0	1	0.7	0	0.0
	Four months	12	3.3	2	2.2	10	6.7	0	0.0
	Five months	85	23.	28	30.	34	22.	23	18.
	Six months	22	60.	54	58.	77	51.	91	73.
	Seven months	25	6.8	8	8.6	8	5.3	9	7.3
	Unknown	2	0.5	0	0.0	2	1.3	0	0.0
Number of months since onset of symptoms**	Less than one month	5	1.5	1	1.1	3	2.2	1	0.9
	One month	8	2.4	0	0.0	8	5.8	0	0.0
	Two months	3	0.9	0	0.0	3	2.2	0	0.0
	Three months	1	0.3	0	0.0	1	0.7	0	0.0
	Four months	12	3.5	2	2.3	10	7.2	0	0.0
	Five months	78	22.	25	28.	32	23.	21	18.
	Six months	21	61.	52	59.	73	52.	85	74.
	Seven months	23	6.8	8	9.1	8	5.8	7	6.1

*Participants were asked which one describes MOST of their current work ** excludes 27 participants who were not symptomatic at the time of their positive PCR test

Appendix 4F Factors associated with Abbott SARS-CoV-2 IgG seronegativity, among participants who had a previously PCR-confirmed infection, including those who had a grayzone result (n=367)**,** PRECISE 1, October 2020

		All (	Negativ e	Negativ e	Unadjusted OR	95% CI	P-valu e	Adjusted	95% CI	P-valu e
Total		3	124	33.8						
Age group	18-29	1	41	36.9	*			*		
	30-39	1	43	39.8	1.13	0.66 –	0.	0.96	0.51 -	0.89
	40-49	7	17	21.5	0.47	0.24 –	0.	0.44	0.21 -	0.03
	50-59	5	19	38.0	1.05	0.52 –	0.	1.05	0.47 -	0.89
	60+	1	4	21.1	0.46	0.12 –	0.	0.32	0.08 -	0.09
Sex	Female	2	102	37.9	*			*		
	Male	**9**	**22**	**22.4**	**0.47**	**0.28 –**	**0.**	**0.38**	**0.21 -**	**0.00**
Ethnicity	Irish	2	94	39.7	*			*		
	Any other white	4	12	27.3	0.57	0.27 –	0.	0.64	0.29 -	0.27
	Any Asian	7	14	19.4	0.37	0.19 –	0.	0.41	0.19 -	0.01
	Any African/black	1	2	20.0	0.38	0.05 –	0.	0.48	0.07 -	0.39
	Other	4	2	50.0	1.52	0.18 –	0.	1.53	0.15 -	0.70
Close contact	Yes	2	65	30.8	0.72	0.46 –	0.	0.67	0.4 -	0.12
	No	1	59	38.3	*			*		
Main type of patient	No patient contact	6	24	39.3	*			*		
	Daily contact non-	2	74	33.9	0.79	0.44 –	0.	0.78	0.4 -	0.48
	Daily contact	8	26	29.5	0.65	0.21 –	0.	0.84	0.38 -	0.67
Severity of symptoms**	No symptoms	7	3	42.9	1.08	0.21 –	0.	1.07	0.17 -	0.94
	Minor symptoms	1	62	41.1	*			*		
	Significant	1	55	30.9	0.64	0.41 –	0.	0.58	0.34 -	0.03
	Severe symptoms	2	4	13.8	0.23	0.06 –	0.	0.26	0.07 -	0.02
HCW Role	Administration	2	9	37.5	*					not entered
	Allied health care	4	19	45.2	1.38	0.49 –	0.			
	General Support	1	5	41.7	1.19	0.28 –	0.			
	Healthcare assistant	3	5	15.6	0.31	0.08 –	0.			
	Medical/Dental	7	29	39.7	1.10	0.43 –	0.			
	Nursing/midwifery	1	55	30.4	0.73	0.31 –	0.			
	Other	3	2	66.7	3.33	0.28 –	0.			
Number of months since PCR positive test**	Less than one month	8	1	12.5	NA	NA	N	0.23	0.01 -	0.20
	One month	8	0	0.0	NA	NA	N	NA	NA	NA
	Two months	4	0	0.0	NA	NA	N	NA	NA	NA
	Three months	1	0	0.0	NA	NA	N	NA	NA	NA
	Four months	1	0	0.0	NA	NA	N	NA	NA	NA
	Five months	8	23	27.1	*			*		
	Six months	2	91	41.0	1.87	1.09 –	0.	1.95	1.07 -	0.03
	Seven months	2	9	36.0	1.52	0.57 –	0.	2.07	0.72 -	0.17

*reference category
P-value calculated using the chi-square test
**Excludes two unknowns

^Participants were asked which type of patient contact describes MOST of their current work (excludes five unknowns)

Table 5A Response rate by HCW type, PRECISE 2, April 2021

HCW role	St James's Hospital response rate			University Hospital Galway response Rate			Combined response rate		
	N	n	%	N	n	%	N	n	%
Admin	693	403	58%	639	273	43%	1332	676	51%
Medical/dental	621	357	57%	814	356	44%	1435	713	50%
Nursing/ midwifery	1802	1097	61%	1689	794	47%	3491	1,891	54%
Allied health	742	612	82%	648	432	67%	1390	1,044	75%
General support	412	243	59%	240	122	51%	652	365	56%
Health care assistant	422	179	42%	316	112	35%	738	291	39%
Other	-	54		-	51		-	105	
Total	4692	2945	63%	4346	2140	49%	9038	5085	56%

Table 5B Comparison of all staff and study participants in St James's Hospital by healthcare worker role, PRECISE 2, April 2021

HCW role	St James's Hospital all staff		St James's Hospital response study participants		Difference	
	N	%	n	%	n	%
Admin	693	15%	403	14%	290	-1.1%
Medical/dental	621	13%	357	12%	264	-1.1%
Nursing/ midwifery	1802	38%	1097	37%	705	-1.2%
Allied health	742	16%	612	21%	130	5.0%
General support	412	8.8%	243	8.3%	169	-0.5%
Health care assistant	422	9.0%	179	6.1%	243	-2.9%
Other	-	-	54	1.8%	-	-
Total	4692	100%	2945	100%	1747	-

Table 5C Comparison of all staff and study participants in University Hospital Galway by healthcare worker role, PRECISE 2, April 2021

HCW role	University Hospital Galway all staff		University Hospital Galway response study participants		Difference	
	N	%	n	%	n	%
Admin	639	15%	273	13%	366	-1.9%
Medical/dental	814	19%	356	17%	458	-2.0%
Nursing/ midwifery	1689	39%	794	37%	895	-1.6%
Allied health	648	15%	432	20%	216	5.3%
General support	240	5.5%	122	5.7%	118	0.2%
Health care assistant	316	7.2%	112	5.2%	204	-2.0%
Other	-	-	51	2.4%	-	-
Total	4364	100%	2140	100%	2224	-

Table 5D Comparison of all staff and study participants by healthcare worker role, PRECISE 2, April 2021

HCW role	All invited staff		All study participants		Difference	
	N	%	n	%	n	%
Admin	1332	15%	676	13%	656	-1.5%
Medical/dental	1435	16%	713	14%	722	-1.9%
Nursing/ midwifery	3491	39%	1,891	37%	1600	-1.5%
Allied health	1390	15%	1,044	21%	346	5.1%
General support	652	7.2%	365	7.2%	287	0.0%
Health care assistant	738	8.2%	291	5.7%	447	-2.5%
Other		-	105	2.1%	-	-
Total	9028	100%	5085	100%	3943	-

Table 5E Characteristics of HCW with SARS-CoV-2 seropositivity (n=898), by hospital, PRECISE 2, April 2021

Participant characteristics		St James's Hospital Dublin		University Hospital Galway		Both Hospitals	
		n	%	n	%	n	%
Overall		623	21%	275	13%	898	18%
Median age (IQR)		39 (29-49)		35 (27-44)		38 (29-48)	
Age groups (years)	18-29	159	26%	90	33%	249	28%
	30-39	154	25%	84	31%	238	27%
	40-49	157	25%	51	19%	208	23%
	50-59	119	19%	39	14%	158	18%
	Over 60	34	5.5%	11	4.0%	45	5.0%
Sex	Female	471	76%	198	72%	669	74%
	Male	152	24%	77	28%	229	26%
Ethnicity	Irish	401	64%	194	71%	595	66%
	Any other white background	56	9.0%	38	14%	94	10%
	Asian background	122	20%	26	9.5%	148	16%
	African/other black background	29	4.7%	10	3.6%	39	4.3%
	Other	15	2.4%	7	2.5%	22	2.4%
Country of birth	Ireland	386	62%	181	66%	567	63%
	United Kingdom	25	4.0%	19	6.9%	44	4.9%
	India	60	10%	16	5.8%	76	8.5%
	Philippines	53	8.5%	1	0.4%	54	6.0%
	Poland	8	1.3%	12	4.4%	20	2.2%
	USA	4	0.6%	7	2.5%	11	1.2%
	Romania	12	1.9%	3	1.1%	15	1.7%
	Nigeria	18	2.9%	1	0.4%	19	2.1%
	Other	57	9.1%	35	13%	92	10%
Education	Primary	7	1.1%	0	0.0%	7	0.8%
	Secondary	105	17%	28	10%	133	15%
	Third level	301	48%	133	48%	434	48%
	Post-graduate	210	34%	114	41%	324	36%
Role	Admin	64	10%	21	7.6%	85	9.5%
	Medical/dental	55	8.8%	61	22%	116	13%
	Nursing/ midwifery	281	45%	114	41%	395	44%
	Allied health	89	14%	29	11%	118	13%
	General support	60	10%	21	7.6%	81	9.0%
	Health care assistant	70	11%	23	8.4%	93	10%
	Other	4	0.6%	6	2.2%	10	1.1%
Lives with	Alone	42	6.7%	19	6.9%	61	6.8%
	With others	578	92.8%	256	93%	834	92.9%
	Missing	3	0.5%	0	0.0%	3	0.3%
Lives with HCW	Yes	234	38%	106	39%	340	38%
	No	376	60%	164	60%	540	60%
	Missing	13	2.1%	5	1.8%	18	2.0%
Daily contact with COVID-19 patients	Contact with COVID-19 patients	196	31%	69	25%	265	30%
	Contact with patients without COVID-19	293	47%	170	62%	463	52%
	No patient contact	134	22%	36	13%	170	19%

Previous COVID-19 symptoms	No symptoms	121	19%	48	17%	169	19%
	Had symptoms	502	81%	227	83%	729	81%
	Missing	0	0.0%	0	0.0%	0	0.0%
Severity of symptoms	No symptoms	121	19%	48	17%	169	19%
	Mild symptoms	228	37%	107	39%	335	37%
	Significant symptoms	262	42%	102	37%	364	41%
	Severe (hospitalised)	12	1.9%	18	6.5%	30	3.3%
	Missing	0	0.0%	0	0.0%	0	0.0%
Previous positive COVID-19 PCR test	No	190	30%	45	16%	235	26%
	Yes	433	70%	230	84%	663	74%
Symptoms at time of previous positive PCR test	No	51	12%	36	16%	87	13%
	Yes	382	88%	194	84%	576	87%
Severity of symptoms at time of PCR test	No symptoms	51	12%	36	16%	87	13%
	Mild symptoms	143	33%	83	36%	226	34%
	Significant symptoms	227	52%	93	40%	320	48%
	Severe (hospitalised)	12	2.8%	18	7.8%	30	4.5%
	Missing	0	0.0%	0	0.0%	0	0.0%

Table 13e Prevalence of SARS-CoV-2 seropositivity by participant characteristics, both hospitals, PRECISE 2, April 2021

Participant characteristics		Total	SARS-CoV-2 seropositive		
		N	**n**	**% (95% CI)**	**p-value***
Overall		5085	898	18 (17 - 19)	
Hospital	St James's Hospital	2,945	623	21 (20 - 23)	<0.001
	University Hospital Galway	2,140	275	13 (11 - 14)	
Age groups (years)	18-29	1108	249	22 (20 - 25)	<0.001
	30-39	1330	238	18 (16 - 20)	
	40-49	1414	208	15 (13 - 17)	
	50-59	951	158	17 (14 - 19)	
	Over 60	282	45	16 (12 - 21)	
Sex	Female	3,959	669	17 (16 - 18)	0.008
	Male	1,126	229	20 (18 - 23)	
Ethnicity	Irish	3,798	595	16 (15 - 17)	<0.001
	Any other white background	476	94	20 (16 - 24)	
	Asian background	599	148	25 (21 - 28)	
	African or any other black background	117	39	33 (25 - 43)	
	Other	95	22	23 (15 - 33)	
Country of birth	Ireland	3,630	567	16 (14 - 17)	<0.001
	United Kingdom	295	44	15 (11 - 20)	
	India	293	76	26 (21 - 31)	
	Philippines	214	54	25 (20 - 32)	
	Poland	85	20	24 (15 - 34)	
	USA	55	11	20 (10 - 33)	
	Romania	45	15	33 (20 - 49)	
	Nigeria	35	19	54 (31 - 71)	
	Other	433	92	21 (17 - 25)	
Education	Primary	22	7	32 (14 - 55)	<0.001
	Secondary	609	133	22 (19 - 25)	
	Third level	2,244	434	19 (18 - 21)	
	Post-graduate	2,210	324	15 (13 - 16)	
Role	Admin	676	85	13 (10 - 15)	<0.001
	Medical/dental	713	116	16 (14 - 19)	
	Nursing/ midwifery	1,891	395	21 (19 - 23)	
	Allied health	1,044	118	11 (9.4 - 13)	
	General support	365	81	22 (18 - 27)	
	Health care assistant	291	93	32 (27 - 38)	
	Other	105	10	10 (4.7 - 17)	
Lives with	Alone	464	61	13 (10 - 17)	0.008
	With others	4,610	834	18 (17 - 19)	
	Missing	11	3	27 (6.0 - 61)	
Lives with HCW	Yes	1,571	340	22 (20 - 24)	<0.001
	No	3412	540	16 (16 - 17)	
	Missing	102	18	18 (11 - 26)	

*Calculated using the chi-square test

Table 13f Prevalence of SARS-CoV-2 seropositivity by COVID-19 related characteristics, both hospitals, PRECISE 2, April 2021

COVID-19 related characteristics		Total	SARS-CoV-2 seropositive		
		N	**n**	**% (95% CI)**	**p-**
Daily contact with COVID-19	Contact with COVID-19 patients	1136	265	23 (21 - 26)	<0.001
	Contact with patients without COVID-19	2,500	463	19 (17 - 20)	
	No patient contact	1,449	170	12 (10 - 14)	
Previous COVID-19 symptoms	No symptoms	2913	169	5.8 (5.0 - 6.7)	<0.001
	Had symptoms	2171	729	34 (32 - 36)	
	Missing	1	0	-	
Severity of symptoms	No symptoms	2913	169	5.8 (5.0 - 6.7)	<0.001
	Mild symptoms	1502	335	22 (20 - 24)	
	Significant symptoms	621	364	59 (55 - 63)	
	Severe (hospitalised)	47	30	64 (49 - 77)	
	Missing	1	0	-	
Previous positive COVID-19 PCR test	No	4273	235	5.5 (4.8 - 6.2)	<0.001
	Yes	812	663	82 (79 - 84)	
Symptoms at time of previous positive PCR test	No	172	87	51 (43 - 58)	<0.001
	Yes	640	576	90.0 (87 - 92.2)	
Severity of symptoms at time of PCR test	No symptoms	172	87	51 (43 - 58)	<0.001
	Mild symptoms	256	226	88 (84 - 92.0)	
	Significant symptoms	351	320	91.2 (88 -	
	Severe (hospitalised)	32	30	93.8 (79 -	
	Missing	1	0	-	

*Calculated using the chi-square test

Table 13g Prevalence of SARS-CoV-2 seropositivity for general support staff, by role and hospital, PRECISE 2, April 2021

Role	**St James's Hospital**			**University Hospital Galway**			**Both hospitals**		
	Total	**Seropositive**		**Total**	**Seropositive**		**Total**	**Seropositive**	
	N	**n**	**%**	**N**	**n**	**%**	**N**	**n**	**%**
Domestic/ Cleaning	62	15	24%	45	9	20%	107	24	22%
Catering	83	23	28%	18	5	28%	101	28	28%
Maintenance	27	6	22%	24	1	4.2%	51	7	14%
Security	33	6	18%	14	3	21%	47	9	19%
Porter	20	6	30%	13	1	7.7%	33	7	21%
Chaplain	11	3	27%	2	0	0.0%	13	3	23%
Other	7	1	14%	0	0	-	7	1	14%
Driver	0	0	-	6	2	33%	6	2	33%
Total	**243**	**60**	**25%**	**122**	**21**	17%	**365**	**81**	**22%**

Table 13h Prevalence of asymptomatic SARS-CoV-2 infection, by hospital location and ethnicity, PRECISE 2, April 2021

		Total seropositive	Asymptomatic SARS-CoV-2 infection		
		N	**n**	**% (95% CI)**	**p-value***
St James's Hospital	Total	623	121	19 (16 - 23)	0.009
	Irish	401	82	20 (17 - 25)	
	Other white background	56	13	23 (13 - 36)	
	Asian	122	13	11 (5.8 - 18)	
	African or other black	29	11	38 (21 - 58)	
	Other	15	2	13 (1.7 - 40)	
Galway University Hospital	Total	275	48	17 (13 - 22)	0.531
	Irish	194	35	18 (13 - 24)	
	Other white background	38	5	13 (4.4 - 28)	
	Asian	26	5	19 (6.6 - 39)	
	African or other black	10	3	30 (6.7 - 65)	
	Other	7	0	-	
Both hospitals	Total	898	169	19 (16 - 22)	0.010
	Irish	595	117	20 (17 - 23)	
	Other white background	94	18	19 (12 - 29)	
	Asian	148	18	12 (7.4 - 19)	
	African or other black	39	14	36 (21 - 53)	
	Other	22	2	9.1 (1.1 - 29)	

*Calculated using the Chi-square test

Table 13i Undiagnosed SARS-CoV-2 infection, by HCW role and hospital location, PRECISE 2, April 2021

	St James's Hospital		University Hospital Galway		Both hospitals	
	n	**%**	**n**	**%**	**n**	**%**
Admin	19	10%	5	11%	24	10%
Medical/dental	12	6.3%	8	18%	20	8.5%
Nursing/ midwifery	79	42%	19	42%	98	42%
Allied health	32	17%	6	13%	38	16%
General support	22	12%	4	8.8%	26	11%
Health care assistant	26	14%	2	4.4%	28	12%
Other	0	0.0%	1	2.2%	1	0.4%
Total	190	100%	45	100%	235	100%

Table 5F. Characteristics of fully vaccinated participants with PCR confirmed infection i.e., vaccine breakthrough cases, both hospitals (n=23), PRECISE 2, April 2021

Participant characteristics		**PCR positive ≥14 days after second vaccine dose (N=23)**	
		n	**%**
Hospital	St James's Hospital	18	78%
	University Hospital Galway	5	22%
Age groups	18-29	2	8.7%
	30-39	6	26%
	40-49	8	35%
	50-59	5	22%
	≥60	2	8.7%
Sex	Female	15	65%
	Male	8	35%
Ethnicity	Irish	12	52%
	Any other white background	1	4.3%
	Asian background	8	35%
	African or other black background	2	8.7%
	Other	0	0.0%
Country of birth	Ireland	12	52%
	United Kingdom	0	0.0%
	India	4	17%
	Philippines	4	17%
	Poland	1	4.3%
	USA	0	0.0%
	Other	0	0.0%
Education	Primary	0	0.0%
	Secondary	2	8.7%
	Third level	11	48%

	Post-graduate	10	43%
Role	Admin	0	0.0%
	Medical/dental	0	0.0%
	Nursing/ midwifery	10	43%
	Allied health	5	22%
	General support	2	8.7%
	Health care assistant	4	17%
	Other	2	8.7%
Workplace exposure to COVID-19 patients	No patient contact	2	8.7%
	Daily contact with COVID-19 patients	9	39%
	Daily contact with patients without COVID-19	12	52%
Lives with	Alone	1	4.3%
	With others	22	96%
	Missing	0	0.0%
Lives with HCW	Yes	13	57%
	No	10	43%
Vaccine type	Pfizer	23	100%
	Other	0	0.0%
Symptoms at the time of positive PCR test	Yes	5	22%
	No	18	78%

Table 6A Characteristics of participants who took part in both PRECISE 1 (October 2020) and PRECISE 2 (April 2021)

Participant characteristics*		**Total (N=3,313)**	
		N	**%**
Age group	18-29	633	19
	30-39	856	26
	40-49	967	29
	50-59	664	20
	≥60	193	5.8
Sex	Female	2,625	79
	Male	688	21
Ethnicity	Irish	2,615	79
	Any other white background	287	8.7
	Any Asian background	314	9.5
	Any African or black background	59	1.8
	Other	38	1.1
Country of birth	Ireland	2487	75
	United Kingdom	222	6.7
	India	154	4.6
	Philippines	112	3.4

	Poland	48	1.4
	USA	33	1.0
	Other	257	7.8
Education	Primary	11	0.3
	Secondary	373	11
	Third level	1407	42
	Post-graduate	1522	46
Role	Admin	450	14
	Medical/dental	422	13
	Nursing/ midwifery	1265	38
	Allied health	740	22
	General support	214	6.5
	Health care assistant	149	4.5
	Other	73	2.2
Lives with	Alone	296	8.9
	With others	3,013	90.9
	Missing	4	0.1
Lives with HCW	Yes	986	30
	No	2,259	68
	Missing	68	2.1
Daily contact with COVID-19 patients	Contact with COVID-19 patients	489	15
	Contact with patients without COVID-19	1,820	55
	No patient contact	1,004	30

*based on answers given in the first phase

Table 6B Characteristics of matched participants who seroconverted between PRECISE 1 (October 2020) and *PRECISE* 2 (April 2021)

Participant characteristics		**Total (N=235)**	
		N	**%**
Age groups	18-29	63	27
	30-39	59	25
	40-49	59	25
	50-59	40	17
	≥60	14	6.0
Sex	Female	187	80
	Male	48	20
Ethnicity	Irish	181	77
	Any other white background	18	7.7
	Any Asian background	20	8.5
	Any African or black background	6	2.6
	Other	10	4.3
Country of birth	Ireland	173	74
	United Kingdom	11	4.7
	India	12	5.1
	Philippines	5	2.1
	Poland	5	2.1
	USA	2	0.9
	Other	27	11
Education	Primary	2	0.9
	Secondary	40	17
	Third level	122	52
	Post-graduate	71	30
Role	Admin	27	11
	Medical/dental	22	9.4
	Nursing/ midwifery	107	46
	Allied health	29	12
	General support	22	9.4
	Health care assistant	24	10
	Other	4	1.7
Lives with	Alone	14	6.0
	With others	221	94
Lives with HCW	Yes	92	39
	No	141	60
	Missing	2	0.9
Daily contact with COVID-19 patients	Contact with COVID-19 patients	43	18
	Contact with patients without COVID-19	144	61
	No patient contact	48	20

*based on answers given in the PRECISE 1

Appendix 2 - Publications based on this research

Appendix 2.1 Allen N et al. *Epidemiology and Infection* (2021)

Epidemiology and Infection

cambridge.org/hyg

Original Paper

Prevalence of antibodies to SARS-CoV-2 in Irish hospital healthcare workers

Niamh Allen[1], Una Ni Riain[2], Niall Conlon[1], Annamaria Ferenczi[3], Antonio Isidro Carrion Martin[4], Lisa Domegan[3], Cathal Walsh[3,5,6], Lorraine Doherty[3], Cliona O'Farrelly[7], Eibhlin Higgins[8], Colm Kerr[1], Jonathan McGrath[1], PRECISE Study Steering Group[†], Lorraine Doherty, Niamh Allen, Colm Bergin, Niall Conlon, Lisa Domegan, Catherine Fleming, Margaret Fitzgerald, Cillian de Gascun, Joan Gallagher, Derval Igoe, Mary Keogan, Noirin Noonan, Cliona O'Farrelly, Una Ni Rian, Breda Smyth, Catherine Fleming[8,*] and Colm Bergin[1,*]

[1]Department of GU Medicine and Infectious Diseases (GUIDE), St. James's Hospital, Dublin, Ireland; [2]Department of Microbiology, University Hospital Galway, Galway, Ireland; [3]Health Protection Surveillance Centre (HPSC), Dublin, Ireland; [4]Department of Public Health, University of Murcia, Murcia, Spain; [5]Health Research Institute and MACSI, University of Limerick, Limerick, Ireland; [6]MISA and NCPE, St James's Hospital, Dublin, Ireland; [7]School of Medicine and School of Biochemistry and Immunology, Trinity College Dublin, Dublin, Ireland and [8]Department of Infectious Diseases, University Hospital Galway, Galway, Ireland

*Supervising authors contributed equally.

Cite this article: Allen N *et al* (2021). Prevalence of antibodies to SARS-CoV-2 in Irish hospital healthcare workers. *Epidemiology and Infection* **149**, e157, 1–11. https://doi.org/10.1017/S0950268821000984

Received: 1 March 2021
Revised: 12 April 2021
Accepted: 21 April 2021

Key words:
Coronavirus; COVID-19; healthcare workers; SARS-CoV-2 antibodies; seroprevalence

Author for correspondence:
Niamh Allen,
E-mail: nallen@stjames.ie, allen.niamh@gmail.com

Abstract

Hospital healthcare workers (HCWs) are at increased risk of contracting COVID-19 infection. We aimed to determine the seroprevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies in HCWs in Ireland. Two tertiary referral hospitals in Irish cities with diverging community incidence and seroprevalence were identified; COVID-19 had been diagnosed in 10.2% and 1.8% of staff respectively by the time of the study (October 2020). All staff of both hospitals ($N = 9038$) were invited to participate in an online questionnaire and blood sampling for SARS-CoV-2 antibody testing. Frequencies and percentages for positive SARS-CoV-2 antibody were calculated and adjusted relative risks (aRR) for participant characteristics were calculated using multivariable regression analysis. In total, 5788 HCWs participated (64% response rate). Seroprevalence of antibodies to SARS-CoV-2 was 15% and 4.1% in hospitals 1 and 2, respectively. Thirty-nine percent of infections were previously undiagnosed. Risk for seropositivity was higher for healthcare assistants (aRR 2.0, 95% confidence interval (CI) 1.4–3.0), nurses (aRR: 1.6, 95% CI 1.1–2.2), daily exposure to patients with COVID-19 (aRR: 1.6, 95% CI 1.2–2.1), age 18–29 years (aRR: 1.4, 95% CI 1.1–1.9), living with other HCWs (aRR: 1.3, 95% CI 1.1–1.5), Asian background (aRR: 1.3, 95% CI 1.0–1.6) and male sex (aRR: 1.2, 95% CI 1.0–1.4). The HCW seroprevalence was six times higher than community seroprevalence. Risk was higher for those with close patient contact. The proportion of undiagnosed infections call for robust infection control guidance, easy access to testing and consideration of screening in asymptomatic HCWs. With emerging evidence of reduction in transmission from vaccinated individuals, the authors strongly endorse rapid vaccination of all HCWs.

†PRECISE Study (Prevalence of COVID-19 in Irish Healthcare Workers) Steering Group Members and Affiliations:
Dr Lorraine Doherty, National Clinical Director for Health Protection, HSE-Health Protection Surveillance Centre (HPSC), Dublin, Ireland, and Chair of Steering Group, Dr Niamh Allen, Consultant Physician in Infectious Diseases and Principal Investigator for PRECISE study, Professor Colm Bergin, Consultant Physician in Infectious Diseases and Site Lead for PRECISE study, St. James's Hospital Dublin, Ireland, Dr Niall Conlon, Consultant Immunologist, St. James's Hospital, Dublin, Ireland, Dr Lisa Domegan, Surveillance Scientist, HSE-HPSC, Ireland, Dr Catherine Fleming, Consultant in Infectious Disease and Site Lead for PRECISE study, Galway University Hospital, Galway, Ireland, Dr Margaret Fitzgerald, National Public Health Lead. National Social Inclusion Office, Dublin, Ireland, Dr Cillian de Gascun, Director, UCD National Virus Reference Laboratory, University College Dublin, Dublin, Ireland, Joan Gallagher, Programme Manager, Office of the National Clinical Director for Health Protection, HSE HPSC, Dublin, Ireland, Dr Derval Igoe, Specialist in Public Health Medicine, HSE HPSC, Dublin, 1, Ireland, Prof. Mary Keogan, Consultant Immunologist Beaumont Hospital & Clinical Lead, National Clinical Programme for Pathology, HSE, Ireland, Dr Noirin Noonan, Consultant in Occupation Medicine, St. James's Hospital, Dublin, Ireland., Professor Cliona O'Farrelly, Chair in Comparative Immunology, Trinity College Dublin, Ireland, Dr Una Ni Rian, Consultant Microbiologist, Galway University Hospital, Galway, Ireland, Dr Breda Smyth, Department of Public Health, HSE West, Ireland.

https://doi.org/10.1017/S0950268821000984 Published online by Cambridge University Press

Background

Healthcare workers (HCWs), and those they live with, are at increased risk of contracting COVID-19 viral infection [1–3]. Raised antibody levels to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are an excellent indicator of COVID-19 infection [4]. To date there are no published literature on the seroprevalence of antibodies to SARS-CoV-2 infection in Irish HCWs, but it is known from surveillance data that a high proportion of the COVID-19 cases notified were HCWs [5]. Understanding the transmission and potential immunity dynamics of SARS-CoV-2 in hospitals in Ireland is key to controlling this pandemic at national and hospital levels and adds valuable information to the growing evidence based on the transmission patterns of COVID-19 among HCWs.

Hospital 1 is a tertiary referral hospital in the south inner city of Dublin, the capital city of Ireland (population 1.2 million) and has almost 4700 employees and just over 1000 beds. From March to May 2020 (first wave of the pandemic in Ireland [6]) 9.6% of the staff of Hospital 1 tested positive for SARS-CoV-2 infection via polymerase chain reaction (PCR), and by the start of October (the start of the second wave of the pandemic in Ireland [6]) 10.2% of staff had tested positive by PCR. Hospital 2 is a comparable tertiary referral hospital with almost 4400 employees and over 500 beds, located in Galway, in the West of Ireland (population 80 000); 1.8% of its HCWs had a PCR-confirmed infection at some stage during the time period from March to May 2020 and this remained at 1.8% until the start of October 2020. Hospital 1 is one of the largest acute hospitals in Dublin city; hospital 2 is the main acute hospital serving the city of Galway. Both hospitals received patients with COVID-19 infection throughout the first wave of the pandemic in Ireland, and breakdown by ward and specialty is similar. The community incidence of COVID-19 infection in County Galway was significantly lower than that in County Dublin during this time period, which covered the first wave of the pandemic in Ireland and the start of the second wave [6]. The community seroprevalence was also significantly lower in the West of Ireland as compared with the greater Dublin area; community seroprevalence was 3.1% for hospital 1 and 0.6% for hospital 2 in June 2020 [7, 8].

The purpose of the study was to determine the prevalence of anti-SARS-CoV-2 antibodies in HCWs in these two hospitals with diverging community and healthcare rates of infection to improve the understanding of HCWs' risk factors (demographic, living arrangements and work-related risks) for SARS-CoV-2 infection and to inform risk reduction activities and help health services to prepare for further waves of the pandemic. The study will be repeated in April 2021 to assess changes in overall seroprevalence, changes in individual serostatus over time and antibody response to COVID-19 vaccination.

Methods

Study design

This is a cross-sectional study of the seroprevalence of circulating antibodies to SARS-CoV-2, carried out from 14th to 23rd October 2020. All staff members of both hospitals were invited to participate in an online self-administered consent process and online questionnaire, followed by blood sampling for SARS-CoV-2 antibody testing. Electronic consent and patient reported outcomes were captured using Castor; an eClinical platform that enables decentralised clinical trials [9]. Technical support and walk-in phlebotomy clinics were provided for participants who had difficulty with the online consent process. Information collected in the questionnaire included demographic information, contact details, place and type of work, level of contact with patients, previous COVID-19 symptoms and testing, history of close contact with a confirmed case of COVID-19 and living arrangements. Blood samples were processed anonymously. All samples were tested on two testing platforms: the Abbott Architect SARS-CoV-2 immunoglobulin (Ig)-G assay and the Roche Elecsys anti-SARS-CoV-2 immunoassay [10–12]. Samples with an index result in the Abbott manufacturer's suggested positive and greyzone underwent additional testing in the National Virus Reference Laboratory (NVRL) using the Wantai SARS-CoV-2 AB ELISA distributed by Fortress Diagnostics [13]. A positive result on any of the three assays was considered a positive result. Results were discussed in person with any participant who requested this.

Statistical analysis

Frequencies and percentages were calculated for socio-demographic, epidemiological and clinical characteristics, including antibody results. Characteristics of those with a positive SARS-CoV-2 antibody result were compared to those with undetectable antibody, using the chi-square test. Univariate logistic regression was used to calculate relative risks along with their 95% confidence intervals (CIs) to assess the association between SARS-CoV-2 antibody result and characteristics of the study participants. Multivariable regression analysis was conducted to control for negative and positive confounding and to calculate adjusted relative risks (aRR). No explicit finite population correction or reweighting was carried out. All analyses were conducted in Stata 16 (StataCorp, 2019; College Station, TX, LLC) and R 4.0.3 (R Core Team, 2020, www.R-project.org/).

Results

Participation rates and demographics

All staff working in both hospitals (9038 people) were invited to participate in the study. In hospitals 1 and 2, 65% (3042/4692) and 63% (2745/4395) of staff participated in both questionnaire and blood sample, respectively.

The socio-demographic characteristics of participants were similar in both hospitals. Seventy-seven percent were female, with a median age of 39.5 years (IQR 30.4–48.9); 5.1% of participants were >60 years of age. Regarding ethnicity, 77% of participants were white Irish, 10% Asian (13% in hospital 1 and 7% in hospital 2), 9.5% other white background (majority born in Poland, USA and UK) and 2% African or any other black background. Ninety-one percent of participants live with others, and 31% live with other HCWs. The majority (36%) of participants were nursing staff, 19% were allied health care staff, 17% medical/dental staff, 13% administration staff, 7.5% general support staff, 5% healthcare assistants (HCAs) and 2% other HCWs, broadly reflecting the HCW breakdown of the hospital staff (Table 1). Participation rates among staff groupings were also similar in both hospitals; nurses and HCA were slightly underrepresented at 59% and 39% uptake respectively. In all other groups participation rates were above 60%.

https://doi.org/10.1017/S0950268821000984 Published online by Cambridge University Press

Table 1. Participant characteristics by hospital and total number of participants

	Hospital 1 ($N = 3042$)		Hospital 2 ($N = 2745$)			Total ($n = 5788$)	
Participant characteristics	*n*	%	*n*	%	*P* value[a]	*N*	%
Age groups							
18–29	728	24	632	23	0.717	1350	23
30–39	831	27	785	29		1617	28
40–49	793	26	722	26		1515	26
50–59	532	18	468	17		1001	17
Over 60	158	5.2	146	5.3		304	5.3
Sex							
Female	2326	77	2152	78	0.117	4478	77
Male	716	24	592	22		1308	23
Missing	–	–	1	0.04		1	0.02
Ethnicity							
Irish	2262	74	2182	80		4444	77
Any other white background	267	8.8	284	10	<0.001	551	10
Any Asian background	393	13	184	7	<0.001	577	10
Any African or black background	65	2.1	48	1.8		113	2.0
Other	55	1.8	46	1.7		101	1.8
Missing	–	–	1	0.04		1	0.02
Country of birth[a]							
Ireland	2182	72	2091	76		4273	74
Country of birth[a]							
UK	152	5.0	192	7.0		344	5.9
India	201	6.6	98	3.6		299	5.2
Philippines	166	5.5	25	0.9		191	3.3
Poland	24	0.8	48	1.8		72	1.2
USA	22	0.7	38	1.4		60	1.0
Other	295	9.7	253	9.2		548	9.5
Education							
Primary	27	0.9	2	0.1	<0.001	29	0.5
Secondary	420	14	264	10		684	12
Third level	1300	43	1245	45		2545	44
Post-graduate	1295	43	1232	45		2527	44
Missing	–	–	2	0.1		2	0.03
Role							
Admin	454	15	349	13	<0.001	803	14
Medical/dental	460	15	522	19		982	17
Nursing/midwifery	1045	34	1019	37		2064	36
Allied health	616	20	475	17		1012	19
General support	255	8.4	179	6.5		434	7.5
Health care assistant	157	5.2	129	4.7		286	4.9
Other	55	1.8	72	2.6		127	2.2
Lives with							

(Continued)

https://doi.org/10.1017/S0950268821000984 Published online by Cambridge University Press

Table 1. (Continued.)

Participant characteristics	Hospital 1 (*N* = 3042)		Hospital 2 (*N* = 2745)			Total (*n* = 5788)	
	n	%	*n*	%	*P* value[a]	*N*	%
Alone	256	8.4	223	8.1	0.020	479	8.3
Lives with							
With others	2768	91.0	2518	91.7		5286	91.3
Missing	18	0.6	4	0.2		22	0.4
Lives with HCW							
Yes	928	31	839	31	0.983	1767	31
Lives with HCW							
No	2060	68	1859	68		3919	68
Missing	54	1.8	47	1.7		101	1.8

[a]Calculated using the χ^2 test.

Previous testing and COVID-19-related characteristics of the participants

Hospital 1 staff had a higher percentage of previously confirmed COVID-19 infection; 9.6% of participants reported having tested positive at some stage by PCR compared to 2.7% of participants in hospital 2 (Table 2). Table 2 highlights the COVID-19-related characteristics of the participants by hospital.

Seroprevalence of antibodies to SARS-CoV-2

In hospital 1, the overall seroprevalence of antibodies to SARS-COV-2 was 15% (464/3042). Regarding the level of patient contact, the seroprevalence was 21% (108/510) in participants reporting daily contact with patients with known or suspected COVID-19 infection (we defined this as the high-risk group), 17% (269/1611) in those who reported daily contact with patients without known or suspected COVID-19 infection (intermediate-risk group) and 9.5% (87/918) in those who reported little or no patient contact (low-risk group). In hospital 2, the overall seroprevalence of antibodies to SARS-COV-2 was 4.1% (112/2745); 7.1% (28/392) in the high-risk group; 4.6% (75/1634) in the intermediate-risk group and 1.3% (9/717) in the low-risk group.

When looking at seroprevalence by role, the combined data for both hospitals showed the highest seroprevalence in HCAs, with 18% of those participating in the study having detectable antibodies. This was followed by nurses at 13% and medical/dental staff at 10%. The group with the lowest seroprevalence was the administration staff at 6%. Figure 1 shows the proportion of each staff group with detectable antibodies by hospital.

SARS-CoV-2 antibody and previous diagnosis and symptoms

Ninety-five percent (350/367) of those who had previously confirmed infection by PCR had detectable antibodies. In total, 227/576 (39%) of those with positive antibodies had not previously been diagnosed with COVID-19 infection; this represented 3.9% of all participants. Although 63% (142/227) of these participants with a previously undiagnosed infection reported having had symptoms at some stage, it is impossible to know if these symptoms coincided with the time of undiagnosed infection. Sixteen percent (90/576) of those with detectable antibodies had experienced no symptoms consistent with COVID-19.

Risk factors for antibody positivity to SARS-CoV-2

Tables 3 and 4 show the prevalence of SARS-CoV-2 IgG antibodies by participant characteristics for both hospitals combined. (For the prevalence of SARS-CoV-2 IgG antibodies by participant characteristics for the individual hospitals see Tables A–D, Supplementary annex).

On multivariable analysis of the combined hospital data the aRR of detectable antibody was higher for the following characteristics: working in hospital 1 (aRR 3.7, 95% CI 3.0–4.5, $P < 0.001$), working as an HCA (aRR 2.0, 95% CI 1.4–3.0, $P = 0.001$), working as a nurse (aRR 1.6, 95% CI 1.1–2.2, $P = 0.007$), daily exposure to patients with confirmed or suspected COVID-19 infection (aRR 1.6, 95% CI 1.2–2.1, $P = 0.002$), daily contact with patients not known or suspected to have COVID-19 infection (aRR 1.4, 95% CI 1.1–1.8, $P = 0.008$), age 18–29 years (aRR 1.4, 95% CI 1.1–1.9, $P = 0.006$), living with others (aRR 1.5, 95% CI 1.0–2.1, $P = 0.048$), living with other HCW (aRR 1.3, 95% CI 1.1–1.5, $P = 0.007$), being of Asian background (aRR 1.3, 95% CI 1.0–1.6, $P = 0.028$) and male sex (aRR 1.2, 95% CI 1.0–1.4, $P = 0.046$) (Table 5). (For multivariable analysis by hospital see Tables A and F, Supplementary annex.)

Discussion

Overall seroprevalence

The seroprevalence between hospitals 1 and 2 differed by four-fold, reflecting the difference in incidence and seroprevalence in the community in the two locations; the seroprevalence in both locations was six times the community seroprevalence [7, 8]. A Swedish study found HCW seroprevalence to be three times higher than the community seroprevalence during the first wave of the pandemic [14]. A Greek study found HCW seroprevalence to be between 10 and 22 times higher than the general population [15]; this was attributed in part to insufficient use/availability of personal protective equipment (PPE) in the hospital setting. Infection prevention and control (IPC) measures were the same in hospitals 1 and 2 (based on national guidelines) and there have been no issues with PPE availability in either of the hospitals involved in our study at any stage thus far during the pandemic. In both hospitals staff were re-deployed to improve the hospital's capacity to deal with the outbreak, however staff were not

https://doi.org/10.1017/S0950268821000984 Published online by Cambridge University Press

Table 2. COVID-19-related characteristics by hospital and total number of participants

	Hospital 1 ($N = 3042$)		Hospital 2 ($N = 2745$)			Total ($N = 5788$)	
Participant characteristics	*n*	%	*N*	%	*P* value[a]	*n*	%
Contact of a COVID-19 case							
Yes	1185	39	519	19	<0.001	1704	30
No	1847	61	2224	81		4071	70
Missing	10	0.3	2	0.1		12	0.2
Setting of close contact							
Contact at work	1039	88	456	88	0.916	1495	88
Contact outside of work	146	12	63	12		209	12
Daily contact with COVID-19 patients							
Contact with COVID-19 patients	510	17	392	14	<0.001	902	16
Contact with patients without COVID-19	1611	53	1634	60		3245	56
No patient contact	918	30	717	26		1635	28
Missing	3	0.1	2	0.1		5	0.1
Previous COVID-19 symptoms							
No symptoms	1359	45	1517	55	<0.001	2876	50
Had symptoms	1683	55	1228	45		2911	50
Severity							
No symptoms	1359	45	1517	55	<0.001	2876	50
Only minor symptoms	1214	40	945	34		2159	37
Significant symptoms	442	15	259	9.4		701	12
Hospitalised	27	0.9	24	0.9		51	0.9
Previous COVID-19 PCR test							
Yes	1685	55	1093	40	<0.001	2778	48
No	1353	45	1650	60		3003	52
Missing	4	0.1	2	0.1		6	0.1
Previous positive COVID-19 PCR test							
Yes	292	9.6	75	2.7	<0.001	367	6.3
No	2746	90.3	2668	97.2		5414	93.6
Missing	4	0.1	2	0.1		6	0.1

[a]Calculated using the χ^2 test.

deployed to areas that would have been outside of their scope of practice, and all staff had training on the correct use of PPE. The seroprevalence in hospital 1 was similar to that found in a recent unpublished study in another hospital in the same city [16], suggesting that one of the main risks for infection in HCWs is the community incidence; a higher community incidence means that HCWs are more likely to be exposed by the nature of their work which involves direct contact with other people, both patients and other HCWs. Although this risk disproportionately affected those with closer patient contact, the risk to HCWs was higher than in the community, even for those who reported little or no patient contact.

The seroprevalence in both hospitals fell within the wide range (1–45%) previously described in other studies [15–21], and fell either side of the European estimate of 8.5% from the meta-analysis published in November 2020 [22].

Previous symptoms and testing

Five percent of participants with a previous PCR-confirmed COVID-19 infection did not have detectable antibodies. The manufacturers' reported test sensitivity is >95% for each assay used [11–13], so while some of these may be false negative results, it is also possible that these participants did not mount an antibody response following infection with COVID-19, or that they had antibody levels below the limits of detection of the test. We feel that a false-positive PCR result is less likely, but also possible.

In both hospitals, the seroprevalence was higher than the known PCR-confirmed diagnoses of COVID-19 infection of the same timeframe (15% *vs.* 10% in hospital 1, and 4.1% *vs.* 1.8% in hospital 2), and was also higher than the self-reported previously confirmed diagnoses (15% *vs.* 9.6% in hospital 1, 4.1% *vs.* 2.7% in hospital 2). Sixteen percent of participants with positive antibodies reported never having experienced symptoms that

https://doi.org/10.1017/S0950268821000984 Published online by Cambridge University Press

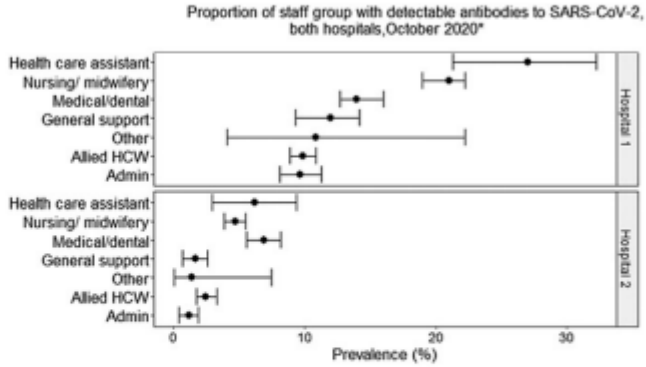


Fig. 1. Proportion of staff group with detectable antibodies to SARS-CoV-2, both hospitals, October 2020.

* finite population correction applied to confidence intervals

were consistent with infection with COVID-19. Thirty-nine percent of infections in our study were undiagnosed, even though both hospitals had onsite PCR testing available to HCWs with symptoms or close contact with a confirmed case of COVID-19 from mid-March 2020. It is likely that these undiagnosed HCWs were working during the infectious period, with potential for onwards transmission to patients and other staff members if proper use of PPE and other IPC measures were not strictly adhered to. This highlights the importance of early detection and reinforces the importance of clear messaging to HCWs about not working when symptomatic. It also highlights the necessity for universal adherence to standard infection control precautions at all times, compliance with transmission-based precautions and appropriate use of PPE including face masks in the hospital setting, irrespective of symptoms [23]. This finding also supports the recommendation for screening of asymptomatic staff when a patient case of hospital-acquired infection, or hospital outbreak of infection with COVID-19 occurs [24]. Mass serial screening of asymptomatic HCWs should be considered. This intervention has been shown to be effective in certain settings [25, 26]. However, the frequency of testing that would be required to have a significant impact on transmission of infection from HCWs has not been established, and other studies have found the impact of this intervention to be uncertain and the logistical challenges it poses to the health service are significant [27–29].

Risk factors for antibody positivity

The main risk factors identified to be significantly associated with SARS-CoV-2 antibody positivity were working in hospital 1, being an HCA, being a nurse, performing roles associated with close patient contact (especially those working directly with COVID-19 patients), living with others, living with other HCWs, being of Asian ethnicity, being aged 18–29 years and being male. Similar risk factors have also been identified in other studies, including the meta-analysis of European studies [22, 30, 31]. Those of Asian background had a higher risk than those of white Irish background. This was a significant finding on multivariable analysis (MVA), with other factors including exposure accounted for. It is possible that there are other social factors relating to ethnicity that were not evaluated in our study and that are contributing to this risk. Studies conducted in other countries have also found Black individuals to be at a higher risk, and many studies have found higher risk in combined BAME (Black, Asian and Minority Ethnic) groups. Black individuals in our study did have a higher overall seroprevalence at 19%, and a higher relative risk of antibody positivity, however this finding was not statistically significant on multivariate analysis, possible due to smaller number of Black participants compared to Asian participants. Other studies have highlighted close patient contact as a risk factor for disease acquisition [32], including specifically the role of nurse or HCAs [15, 31]. We found daily contact with patients with COVID-19 infection to carry a higher relative risk of antibody positivity with comparison to daily contact with patients without COVID-19 infection. Studies have differed on this result; a German study showed a higher seroprevalence among the intermediate risk group with comparison to the high-risk group, potentially due to less scrupulous adherence to infection control precautions including the use of PPE on non-COVID wards [17]. A Spanish study found no significant correlation between role or direct patient contact and antibody positivity, although community incidence was higher in their setting [18].

Having a household contact is known to be a significant risk factor for disease acquisition [33]. In our study, living with others (and especially living with other HCWs) was significantly associated with antibody positivity, which supports the theory that a proportion of the HCW contracting COVID-19 are doing so in their home environment. Other studies have found some correlation between size of household and antibody positivity [18] but to the best of our knowledge this is the first study to find a statistically significant correlation between living with other HCWs and being antibody positive.

Limitations

Our study has several limitations. First, information on COVID-19 symptoms and test results were self-reported and thus could be biased. Second, although the uptake rate of 64% overall is good for an opt-in study, there may be a selection bias; it is possible that those who chose not to take part did so

https://doi.org/10.1017/S0950268821000984 Published online by Cambridge University Press

Table 3. Prevalence of SARS-CoV-2 antibodies by participant characteristics, both hospitals

Participant characteristics	Total *N*	SARS-CoV-2 antibody detected *n*	% (95% CI)	*P* value[a]
Age groups (years)				
18–29	1350	177	13 (11–15)	<0.001
30–39	1617	168	10 (8.9–12)	
40–49	1515	124	8.2 (6.9–9.7)	
50–59	1001	77	7.7 (6.1–9.5)	
Over 60	304	30	9.9 (6.8–14)	
Sex				
Female	4478	422	9.4 (8.6–10)	0.013
Male	1308	154	12 (10–14)	
Ethnicity				
Irish	4444	384	8.6 (7.8–9.5)	<0.001
Any other white background	551	62	11 (8.7–14.2)	
African and any other black background	113	16	14 (8.3–22)	
Asian background	577	107	19 (16–22)	
Other	101	7	6.9 (2.6–15)	
Country of birth[a]				
Ireland	4273	373	8.7 (7.9–9.6)	<0.001
UK	344	32	9.3 (6.5–13)	
India	299	54	18 (14–31)	
Philippines	191	47	25 (19–31)	
Poland	72	10	14 (6.9–24)	
USA	60	3	5.0 (1.0–14)	
Other	548	57	10 (8.0–13)	
Education				
Primary	29	4	14 (3.9–32)	0.055
Secondary	684	61	8.9 (6.9–11)	
Third level	2545	283	11 (9.9–12)	
Post-graduate	2527	228	9.0 (7.9–10)	
Role				
Admin	803	48	6.0 (4.4–7.9)	<0.001
Medical/dental	982	102	10 (8.6–13)	
Nursing/midwifery	2064	263	13 (11–14)	
Allied health	1091	73	6.7 (5.3–8.3)	
General support	434	33	7.6 (5.3–11)	
Health care assistant	286	50	18 (13–22)	
Other	127	7	5.5 (2.2–11)	
Lives with				
Alone	479	28	5.9 (3.9–8.3)	0.007
With others	5286	546	10 (9.5–11)	
Missing	22	2	9.1 (1.1–29)	
Lives with HCW				

(Continued)

https://doi.org/10.1017/S0950268821000984 Published online by Cambridge University Press

Table 3. (*Continued.*)

Participant characteristics	Total *N*	SARS-CoV-2 antibody detected *n*	% (95% CI)	*P* value[a]
Yes	1767	234	13 (12–15)	<0.001
No	3919	332	8.5 (7.6–9.4)	
Missing	101	10	9.9 (4.9–18)	

[a]Calculated using the χ^2 test.

Table 4. Prevalence of SARS-CoV-2 antibodies by COVID-19-related characteristics, both hospitals

Participant characteristics	Total *N*	SARS-CoV-2 IgG detected *n*	% (95% CI)	*P* value[a]
Contact of a COVID-19 case				
Yes	1704	325	19 (17–21)	<0.001
No	249	249	6.1 (5.4–6.9)	
Missing	10	2	167 (2.1–48)	
Setting of close contact				
Contact at work	1495	269	18 (16–20)	0.002
Contact outside of work	209	56	27 (21–33)	
Workplace exposure				
Daily contact with COVID-19 patients	902	136	15 (13–18)	<0.001
Daily contact with patients without COVID	3245	344	11 (9.6–12)	
No patients contact	1635	96	5.9 (4.9–7.1)	
Previous COVID-19-like symptoms				
No symptoms	2876	92	3.2 (2.6–3.9)	<0.001
Had symptoms	2911	484	17 (15–18)	
Previous COVID-19 PCR test				
Yes	2778	474	17 (16–19)	<0.001
No	3003	102	3.4 (2.8–4.1)	
Previous positive COVID-19 PCR test				
Yes	367	350	95.4 (92.7–97.3)	<0.001
No	5414	226	4.2 (3.7–4.7)	

[a]Calculated using the χ^2 test.

due to busier workload, for example, those working on a COVID-19 ward, and therefore with a higher risk of COVID-19 infection. One of the main reasons for overall good recruitment was the incentive of each participant receiving their individual result. This too may introduce a selection bias; those who already know that they have had COVID-19 infection may have been less interested in participating, as well as those who may have already had private antibody testing done elsewhere, which could lead to an under-estimate in the true seroprevalence. Conversely, those who had a previously confirmed infection by PCR may have had more interest in participating to see if they had gained antibodies (and potential immunity). Third, although the communication strategy was an important part of the recruitment process, the study took part during our second wave of the pandemic, and therefore also relied heavily on engagement with information technology (IT) platforms (email, messenger groups and hospital intranet) and less on face-to-face announcements. The online consent process, questionnaire and blood test booking system risks exclusion of those who are less literate in IT. This was identified as a potential limitation from the start, and attempts were made to mitigate this selection bias. Multilanguage information and plain English were used, and groups identified as potentially at risk of exclusion on this basis were targeted directly for inclusion in the study, with small-group sessions to aid consent and questionnaire completion and walk-in clinics for phlebotomy. In the fourth instance, in testing on two different platforms, we chose to prioritise sensitivity over specificity. However, the rate of discordant results was low, and unlikely to have had a significant effect on the results; there were only 21 samples with a positive result on the Abbott Architect assay that did not have a

https://doi.org/10.1017/S0950268821000984 Published online by Cambridge University Press

Table 5. Association between risk factors and the presence of SARS-CoV-2 antibodies, both hospitals

Participant characteristics	Unadjusted relative risk (95% CI)	*P* value	aRR (95% CI)	*P* value
Hospital				
Hospital 2	Ref.			
Hospital 1	**3.7 (3.1–4.6)**	**<0.001**	**3.7 (3.0–4.5)**	**<0.001**
Age groups (years)				
18–29	**1.7 (1.3–2.2)**	**<0.001**	**1.4 (1.1–1.9)**	**0.006**
30–39	1.4 (1.1–1.8)	0.022	1.2 (0.9–1.5)	0.217
40–49	1.1 (0.8–1.4)	0.656	1.0 (0.8–1.3)	0.978
50–59	Ref.			
Over 60	1.3 (0.9–1.9)	0.224	1.4 (0.9–2.0)	0.112
Sex				
Female	Ref.			
Male	**1.3 (1.1–1.5)**	**0.012**	**1.2 (1.0–1.4)**	**0.046**
Ethnicity				
Irish	Ref.			
Any other white background	1.3 (1.0–1.7)	0.041	1.3 (1.0–1.6)	0.068
African and other black background	1.6 (1.0–2.7)	0.037	1.3 (0.8–2.0)	0.299
Asian background	**2.2 (1.8–2.6)**	**<0.001**	**1.3 (1.0–1.6)**	**0.028**
Other	0.8 (0.4–1.7)	0.549	0.6 (0.2–1.3)	0.177
Country of birth				
Ireland	Ref.		Did not enter	
India	2.1 (1.6–2.7)	<0.001		
Philippines	2.8 (2.2–3.7)	<0.001		
UK	1.1 (0.8–1.5)	0.717		
Poland	1.6 (0.9–2.9)	0.119		
USA	0.6 (0.2–1.7)	0.324		
Other	1.2 (0.9–1. 6)	0.193		
Education				
Primary	1.5 (0.6–3.8)	0.365	Did not enter	
Secondary	1.0 (0.8–1.3)	0.933		
Third level	1.2 (1.0–1.5)	0.013		
Post-graduate	Ref.			
Role				
Admin	Ref.			
Doctor/Dental	1.7 (1.3–2.4)	0.001	1.2 (0.8–1.7)	0.327
Nursing	**2.1 (1.6–2.9)**	**<0.001**	**1.6 (1.1–2.2)**	**0.007**
HCA	**2.9 (2.0–4.2)**	**<0.001**	**2.0 (1.4–3.0)**	**0.001**
General support	1.3 (0.8–2.0)	0.270	0.9 (0.6–1.4)	0.687
Allied HCW	1.1 (0.8–1.6)	0.531	0.9 (0.6–1.3)	0.635
Other	0.9 (0.4–2.0)	0.837	1.0 (0.5–2.1)	0.941
Lives with				
Alone	Ref.			
With others	**1.8 (1.2–2.6)**	**0.002**	**1.5 (1.0–2.1)**	**0.048**
Lives with HCW				

(*Continued*)

https://doi.org/10.1017/S0950268821000984 Published online by Cambridge University Press

Table 5. (*Continued.*)

Participant characteristics	Unadjusted relative risk (95% CI)	*P* value	aRR (95% CI)	*P* value
No	Ref.			
Yes	**1.6 (1.4–1.8)**	**<0.001**	**1.3 (1.1–1.5)**	**0.007**
Contact of a COVID-19 case				
No	Ref.		Did not enter	
Yes	3.1 (2.7–3.6)	<0.001		
Close contact at work[a]				
No	1.5 (1.2–1.9)	0.002	Did not enter	
Yes	Ref.			
Workplace exposure to COVID-19 patients				
No patient contact	Ref.			
Daily contact with patients without COVID-19	**1.8 (1.5–2.3)**	**<0.001**	**1.4 (1.1–1.8)**	**0.008**
Daily contact with COVID-19 patients	**2.6 (2.0–3.3)**	**<0.001**	**1.6 (1.2–2.1)**	**0.002**
Previous COVID-19-like symptoms				
No	Ref.		Did not enter	
Yes	5.2 (4.2–6.5)	<0.001		

[a]Calculated for close contacts of COVID-19 cases only (*n* = 1704).

positive result on either the Roche Elecsys assay or the Wantai ELISA. In the fifth instance, this population was surveyed in October, at the start of the second wave of the pandemic in Ireland. A proportion of the HCW workforce in Ireland is transient, and the staff included in the study may not have worked in the same hospital during the first wave of the pandemic. However, over 90% of participants in each hospital reported that had been working in the same hospital during the first wave of the pandemic. And finally, even though the sample size was large, some covariate partners were small and thus rendered further analysis lacking in statistical power and precision (e.g. interactions terms or further stratified analysis).

Conclusion and recommendations

The overall seroprevalence of antibodies to SARS-CoV-2 was 15% in hospital 1 and 4.1% in hospital 2, reflecting the difference in community incidence and seroprevalence in each area, and suggesting that the main risk factor for acquisition of COVID-19 infection in HCWs is the local community incidence. The HCW seroprevalence was six times the community seroprevalence in each area. Specific risk factors for antibody positivity included being an HCA or nurse, daily contact with patients (especially those known or suspected to have COVID-19 infection), age 18–29 years, living with others, in particular living with other HCWs, being of Asian background and being male. The degree of previously undiagnosed and asymptomatic infections highlights the need for ongoing universal adherence to infection control guidance including the use of appropriate PPE in the hospital setting, as well as the importance of early case detection. It is essential that HCWs have easy access to testing, even with mild or no symptoms. As the national COVID-19 vaccination programme is rolled out, we expect that access to testing for HCWs will still be critical. Screening of asymptomatic HCWs in the setting of hospital-acquired patient infection or outbreaks is important and regular screening of asymptomatic HCWs needs to be considered depending on local epidemiology.

This study is a unique comparison between two hospitals in the areas of differing community incidence, in which IPC measures were the same. It is the first study, to the best of our knowledge, to specifically delineate the relationship between living with other HCWs and risk of antibody positivity. This study is paramount in improving understanding of transmission dynamics and HCW risk factors (demographic, workplace- and household-related) in hospitals in Ireland. The high proportion of undiagnosed infections underscores the importance of all interventions to reduce infection in the hospital setting. This bundle should include robust infection control guidance and adherence to that guidance, with scrupulous attention to standard and transmission-based precautions including the use of appropriate PPE in the hospital setting, easy access to testing for HCWs and prompt outbreak investigation. This study will be crucial in informing the vaccination strategy and roll-out of HCWs in Ireland. Emerging evidence of reduced transmission of infection by vaccine recipients [34] endorses prompt vaccination of all HCWs.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0950268821000984

Acknowledgements. We acknowledge the National Public Health Emergency Team (NPHET) who recommended that this study take place, the study steering group who planned the study and critically evaluated this manuscript. The study team who coordinated the running of the study in each hospital, the hospital management at both sites for their support for the study and the staff of both hospitals who participated. We would especially like to acknowledge the phlebotomy departments in each hospital for facilitating the sampling of almost 6000 participants, the microbiology, virology and biochemistry laboratories in each hospital for processing the samples on two different assays, the National Virus Reference Laboratory of Ireland for additional testing and the human resources department for their help with denominator data.

https://doi.org/10.1017/S0950268821000984 Published online by Cambridge University Press

Financial support. This work was supported by the Irish Health Service Executive COVID-19 budget.

Conflict of interest. None of the authors have any conflicts of interest to declare.

Ethical standards. Ethical approval was obtained from the National Research Ethics Committee for COVID-19 in Ireland.

Data availability statement. This dataset is not available to the public for ethical reasons of data protection as certain individuals may be identifiable from the data.

References

1. **Shah ASV, *et al.*** (2020) Risk of hospital admission with coronavirus disease 2019 in healthcare workers and their households: nationwide linkage cohort study. *British Medical Journal* **371**, m3582.
2. **Karlsson U and Fraenkel C-J** (2020) COVID-19: risks to healthcare workers and their families. *British Medical Journal* **371**, m3944.
3. **Richterman A, Meyerowitz EA and Cevik M** (2020) Hospital-acquired SARS-CoV-2 infection: lessons for public health. *Journal of the American Medical Association* **324**, 2155.
4. **Watson J, Richter A and Deeks J** (2020) Testing for SARS-CoV-2 antibodies. *British Medical Journal* **370**, m3325.
5. **HPSC COVID-19 in Healthcare Workers [Internet]** (2021) HPSC COVID-19 in Healthcare Workers. Available at https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/surveillance/covid-19casesinhealthcareworkers/.
6. **HPSC Covid Cases in Ireland [Internet]**. [cited 2021 Jan 13]. Available at https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/casesinireland/epidemiologyofcovid-19inireland/COVID19%20Daily%20infographic.pdf.
7. **Preliminary report of the results of the Study to Investigate COVID-19 Infection in People Living in Ireland (SCOPI): A national seroprevalence study, June-July 2020 [Internet]**. Available at https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/scopi/SCOPI%20report%20preliminary%20results%20final%20version.pdf.
8. **HPSC Surveillance for COVID-19 [Internet]** (2021) HPSC. [cited 2021 Jan 6]. Available at https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/surveillance/.
9. **Castor EDC** (2021) Castor Electronic Data Capture. [online]. Available at https://castoredc.com. [Internet]. 2021. Available at https://www.castoredc.com/.
10. **SARS-CoV-2 IgG Architect - Instructions for Use, FDA [Internet]**. [cited 2020 Dec 14]. Available at https://www.fda.gov/media/137383/download.
11. **ARCHITECT System SARS-COV-2IgG/IgM e- Assay CD-ROM - WW (excluding US) List number 6514-03**. Information update 07th October 2021.
12. **Elecsys® Anti-SARS-CoV-2 Immunoassay for the qualitative detection of antibodies (incl. IgG) against SARS-CoV-2 [Internet]**. [cited 2020 Dec 14]. Available at https://diagnostics.roche.com/global/en/products/params/elecsys-anti-sars-cov-2.html.
13. **WANTAI SARS-CoV-2 Ab ELISA - FDA [Internet]**. Available from https://www.fda.gov/media/140929/download
14. **Rudberg A-S *et al.*** (2020) SARS-CoV-2 exposure, symptoms and seroprevalence in healthcare workers in Sweden. *Nature Communications* **11**, 5064.
15. **Psichogiou M *et al.*** (2020) Antibodies against SARS-CoV-2 among health care workers in a country with low burden of COVID-19. *PLoS One* **15**, e0243025.
16. **Nearly one-fifth of Tallaght hospital staff found to have coronavirus antibodies**. [cited 2020 Dec 12]; Available at https://www.thejournal.ie/tallaght-hospital-staff-antibody-study-5245379-Oct2020/.
17. **Korth J *et al.*** (2020) SARS-CoV-2-specific antibody detection in healthcare workers in Germany with direct contact to COVID-19 patients. *Journal of Clinical Virology* **128**, 104437.
18. **Garcia-Basteiro AL *et al.*** (2020) Seroprevalence of antibodies against SARS-CoV-2 among health care workers in a large Spanish reference hospital. *Nature Communications* **11**, 3500.
19. **Jespersen S *et al.*** (2020) SARS-CoV-2 seroprevalence survey among 17,971 healthcare and administrative personnel at hospitals, pre-hospital services, and specialist practitioners in the central Denmark region. *Clinical Infectious Diseases*, ciaa1471.
20. **Steensels D *et al.*** (2020) Hospital-wide SARS-CoV-2 antibody screening in 3056 staff in a tertiary center in Belgium. *Journal of the American Medical Association* **324**(2), 195–197.
21. **Houlihan C *et al.*** (2020) SARS-CoV-2 virus and antibodies in front-line health care workers in an acute hospital in London: preliminary results from a longitudinal study. *Infectious Diseases (except HIV/AIDS)*. [cited 2020 Jul 24]. medRxiv 2020.06.08.20120584. Available at http://medrxiv.org/lookup/doi/10.1101/2020.06.08.20120584.
22. **Galanis P *et al.*** (2020) Seroprevalence of SARS-CoV-2 antibodies and associated factors in health care workers: a systematic review and meta-analysis. *Journal of Hospital Infection* **108**, 120–134, February 01, 2021.
23. **HPSC Infection Prevention and Control Precautions for COVID-19 [Internet]**. [cited 2021 Jan 12]. Available at https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/guidance/infectionpreventionandcontrolguidance/.
24. **Brown CS *et al.*** (2020) Snapshot PCR surveillance for SARS-CoV-2 in hospital staff in England. *Journal of Infection* **81**, 427–434.
25. **Rivett L *et al.*** (2020) Screening of healthcare workers for SARS-CoV-2 highlights the role of asymptomatic carriage in COVID-19 transmission. *eLife* **9**, e58728.
26. **Moynan D *et al.*** (2020) The role of healthcare staff COVID-19 screening in infection prevention & control. *Journal of Infection* **81**, e53–e54.
27. **Treibel TA *et al.*** (2020) Asymptomatic health-care worker screening during the COVID-19 pandemic – authors' reply. *The Lancet* **396**, 1394–1395.
28. **Chow A *et al.*** (2020) Asymptomatic health-care worker screening during the COVID-19 pandemic. *The Lancet* **396**, 1393–1394.
29. **HPSC General guide on management of COVID-19 outbreaks in the workplace [Internet]**. [cited 2021 Jan 12]. Available at https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/guidance/outbreakmanagementguidance/Guidance%20of%20work%20place%20outbreaks.pdf.
30. **Aldridge RW *et al.*** (2020) Black, Asian and minority ethnic groups in England are at increased risk of death from COVID-19: indirect standardisation of NHS mortality data. *Wellcome Open Research* **5**, 88.
31. **Martin CA *et al.*** (2020) Demographic and occupational determinants of anti-SARS-CoV-2 IgG seropositivity in hospital staff. *Journal of Public Health (Oxford)* , fdaa199.
32. **Lai X *et al.*** (2020) Coronavirus disease 2019 (COVID-2019) infection among health care workers and implications for prevention measures in a tertiary hospital in Wuhan, China. *Journal of the American Medical Association Network Open* **3**, e209666–e209666.
33. **Kumar N *et al.*** (2021) Seroprevalence of antibodies against SARS-CoV-2 among health care workers in Mumbai, India. *Asia Pacific Journal of Public Health* **33**(1), 126–128.
34. **Voysey M, *et al.*** Single Dose Administration, and the Influence of the Timing of the Booster Dose On Immunogenicity and Efficacy of ChAdOx1 nCoV-19 (AZD1222) Vaccine. Available at https://ssrn.com/abstract=3777268.

https://doi.org/10.1017/S0950268821000984 Published online by Cambridge University Press

Appendix 2.2 Allen N et al. *Journal of Infection* (2021), Letter to the Editor

ARTICLE IN PRESS

JID: YJINF [m5G;August 16, 2021;9:47]

Journal of Infection xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Letter to the Editor

Serological markers of SARS-CoV-2 infection; anti-nucleocapsid antibody positivity may not be the ideal marker of natural infection in vaccinated individuals

Dear Editor,

We write in response to correspondence from Whitaker et al. in this Journal (1). The authors demonstrate increases in seropositivity for SARS-CoV-2 antibodies against the spike protein as the roll out of COVID-19 vaccines continues, whilst parallel assessment of nucleocapsid antibodies remained stable. This study is an example of the use of parallel assessment of spike (S) and nucleocapsid (N) antibodies to discriminate between natural infection and vaccine related seropositivity (2) (3). This approach remains attractive, but as the pandemic rolls on it is worth considering the paucity of evidence about the impact of vaccination on antibody production in response to a subsequent natural infection.

As part of a large seroprevalence study in hospital healthcare workers (PRECISE 2 (4)), we measured antibody response following vaccination in over 4000 hospital healthcare workers (HCW), coupled with a questionnaire about previous symptoms and confirmed infection. We measured anti-S and anti-N antibodies using Roche Elecsys total antibody assays to determine both serological response to vaccination and to natural infection. Twenty-three participants reported a breakthrough infection post-vaccination, defined as PCR-confirmed SARS-CoV-2 infection ≥14 days after completion of vaccination (5). This represented 0.6% (23/4111) of all fully vaccinated participants in the study. All had received Pfizer vaccine (which was the vaccine received by most study participants). For these 23 participants, the median number of days between second vaccine dose and positive PCR was 30 days (IQR 25–50 days). Five (22%) had symptoms at the time of the positive PCR test and 18 (78%) did not have symptoms (they were tested as close contacts or as part of hospital outbreaks). All 23 participants had detectable anti-S antibodies, as expected post vaccination (6). Notably, only 6/23 (26%, 95%CI: 11–49) had detectable anti-N antibodies in response to their infection, compared to 663/812 (82%, 95%CI: 79–84, p-value= <0.001 (Chi-squared) of all participants in the study with previous PCR-confirmed infection having detectable anti-N antibodies. Of the 17 that were anti-N negative, median number of days between PCR positivity and sampling mid-point was 52 (range 9–67) so it is surprising that the majority of these had not mounted an anti-N antibody response (7). This low number of seroconversions might suggest that anti-N antibodies may be insensitive as a marker of natural infection post vaccination. It is possible that early viral neutralisation, perhaps even at mucosal surfaces, might modify the natural humoral response and limit the development of anti-N antibodies.

There are a very limited number of studies evaluating the production of anti-N post infection in vaccinated individuals; Demmer et al., in a study awaiting peer-review, have commented on high sensitivity and specificity of anti-N antibody assays in detecting infection in vaccinated individuals (8), however the infection in these individuals pre-dated their vaccination. Studies such as that conducted by Whittaker et al. that we are here responding to, are continuing to rely on anti-N as a marker of seropositivity related to natural infection, including in vaccinated individuals (1). Vaccine effectiveness studies will also incorporate periodic serological testing for SARS-CoV-2 antibodies as a marker of breakthrough infection (9). To the best of our knowledge there are no published data to date that have identified a comparative reduction in anti-N seroconversion following natural infection in vaccinated individuals. Whilst our numbers are small, even in large seroprevalence studies numbers of breakthrough infections will be small (5), and further research of individuals with well-defined vaccine breakthrough infections are required. This information will be critical in informing optimal assessment of seroprevalence in vaccinated cohorts.

References

1. Whitaker HJ, Elgohari S, Rowe C, Otter AD, Brooks T, Linley E, et al. Impact of COVID-19 vaccination program on seroprevalence in blood donors in England, 2021. J Infect 2021 S0163445321002243.
2. Okba NMA, Müller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. Severe acute respiratory syndrome coronavirus 2–Specific antibody responses in coronavirus disease patients. Emerg Infect Dis 2020;26(7):1478–88.
3. Dörschug A, Frickmann H, Schwanbeck J, Yilmaz E, Meese R, Hahn A, et al. Comparative assessment of sera from individuals after S-Gene RNA-based SARS-CoV-2 vaccination with spike-protein-based and nucleocapsid-based serological assays. Diagnostics 2021;11(3):426.
4. Prevalence of antibodies to SARS-CoV-2 natural infection and post-vaccination in Irish Hospital Healthcare Workers (PRECISE 2) [Internet]. Health Protection Surveillance Centre of Ireland (HPSC); 2021 [cited 2021 Jul 26]. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/research/precise/PRECISE%202%20Report.pdf
5. CDC morbidity and mortality weekly report (MMWR) - interim estimates of vaccine effectiveness of BNT162b2 and mRNA-1273 COVID-19 vaccines in preventing SARS-CoV-2 infection among health care personnel, first responders, and other essential and frontline workers — Eight U.S. locations, December 2020–March 2021 [Internet]. 2021 [cited 2021 Jun 16]. Available from: https://www.cdc.gov/mmwr/volumes/70/wr/mm7013e3.htm
6. Eyre DW, Lumley SF, Wei J, Cox S, James T, Justice A, et al. Quantitative SARS-CoV-2 anti-spike responses to Pfizer-BioNTech and Oxford-AstraZeneca vaccines by previous infection status. Clin Microbiol Infect 2021 S1198743X21002895.
7. Algassim A, Alfaleh MA, Hala S, Almajhdi FS, Alamri SS, Almahboub SA, et al. SARS-CoV-2 S1 and N-based serological assays reveal rapid seroconversion and induction of specific antibody response in COVID-19 patients. Sci Rep 2020;10(1):16561.
8. Demmer RT, Baumgartner B, Wiggen TD, Ulrich AK, Strickland AJ, Naumchik BM, et al. Identification of natural SARS-CoV-2 infection in seroprevalence studies among vaccinated populations [Internet]. Infect Dis (except HIV/AIDS) 2021. [cited 2021 Jul 8]. Available from http://medrxiv.org/lookup/doi/10.1101/2021.04.12.21255330 .
9. World Health Organisation: evaluation of COVID-19 vaccine effectiveness [Internet]. 2021 [cited 2021 Jul 8]. Available from: https://www.who.int/publications/i/item/WHO-2019-nCoV-vaccine_effectiveness-measurement-2021.1

https://doi.org/10.1016/j.jinf.2021.08.012

Niamh Allen*
Department of GU Medicine and Infectious Diseases (GUIDE), St. James's Hospital, Dublin, Ireland

Melissa Brady
European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control, (ECDC), Stockholm, Sweden
Health Protection Surveillance Centre (HPSC), Dublin, Ireland

Antonio Isidro Carrion Martin
Division of Preventive Medicine and Public Health, Department of Public Health Sciences, University of Murcia School of Medicine, Murcia, Spain

Lisa Domegan
Health Protection Surveillance Centre (HPSC), Dublin, Ireland

Cathal Walsh
Health Protection Surveillance Centre (HPSC), Dublin, Ireland
Health Research Institute and MACSI, University of Limerick, Ireland
MISA and NCPE, St James's Hospital, Dublin, Ireland

Lorraine Doherty
Health Protection Surveillance Centre (HPSC), Dublin, Ireland

Una Ni Riain
Department of Microbiology, University Hospital Galway, Ireland

Colm Bergin
Department of GU Medicine and Infectious Diseases (GUIDE), St. James's Hospital, Dublin, Ireland

Catherine Fleming
Department of Infectious Diseases, University Hospital Galway, Ireland

Niall Conlon
Department of Immunology, St. James's Hospital, Dublin, Ireland

*Corresponding author.
E-mail address: nallen@stjames.ie (N. Allen)

Antonio Isidro Carrion Martin, Lisa Domegan, Cathal Walsh, Lorraine Doherty, Una Ni Riain, Colm Bergin, Catherine Fleming, Niall Conlon. Serological markers of SARS-CoV-2 infection; anti-nucleocapsid antibody positivity may not be the ideal marker of natural infection in vaccinated individuals, Journal of Infection, 2021, ISSN 0163-4453, https://doi.org/10.1016/j.jinf.2021.08.012.
(https://www.sciencedirect.com/science/article/pii/S0163445321003947)

Appendix 2.3 Allen N et al. *Microbiology Spectrum* (2021)

AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Spectrum

RESEARCH ARTICLE

SARS-CoV-2 Antibody Testing in Health Care Workers: A Comparison of the Clinical Performance of Three Commercially Available Antibody Assays

Niamh Allen,[a] Melissa Brady,[b,c] Antonio Isidro Carrion Martin,[d] Lisa Domegan,[b] Cathal Walsh,[b,e,f] Elaine Houlihan,[g] Colm Kerr,[a] Lorraine Doherty,[b] Joanne King,[h] Martina Doheny,[i] Damian Griffin,[i] Maria Molloy,[j] Jean Dunne,[k] Vivion Crowley,[l] Philip Holmes,[l] Evan Keogh,[l] Sean Naughton,[l] Martina Kelly,[m] Fiona O'Rourke,[m] Yvonne Lynagh,[m] Brendan Crowley,[m] Cillian de Gascun,[n] Paul Holder,[a] Colm Bergin,[a,o] Catherine Fleming,[p] Una Ni Riain,[q] Niall Conlon,[c,o] on behalf of the PRECISE Study Steering Group

[a]Department of GU Medicine and Infectious Diseases, St. James's Hospital, Dublin, Ireland
[b]Health Service Executive-Health Protection Surveillance Centre (HPSC), Dublin, Ireland
[c]European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
[d]Department of Public Health, University of Murcia, Murcia, Spain
[e]Health Research Institute and MACSI, University of Limerick, Limerick, Ireland
[f]MISA and NCPE, St James's Hospital, Dublin, Ireland
[g]Department of Microbiology, University Hospital Galway, Galway, Ireland
[h]Department of Virology, University Hospital Galway, Galway, Ireland
[i]Department of Clinical Biochemistry, University Hospital Galway, Galway, Ireland
[j]Galway University Hospital Group, Galway, Ireland
[k]Department of Immunology, St. James's Hospital, Dublin, Ireland
[l]Department of Biochemistry, St. James's Hospital, Dublin, Ireland
[m]Department of Virology, St. James's Hospital, Dublin, Ireland
[n]National Virus Reference Laboratory, Dublin, Ireland
[o]Department of Clinical Medicine, Trinity College, Dublin, Ireland
[p]Department of Infectious Diseases, University Hospital Galway, Galway, Ireland

Niamh Allen and Melissa Brady contributed equally to this article. Niamh Allen was named first as she was the principal investigator for the study, she instigated and organized this research, and she took the lead in coordinating the analysis and writing the manuscript.

ABSTRACT Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies are an excellent indicator of past COVID-19 infection. As the COVID-19 pandemic progresses, retained sensitivity over time is an important quality in an antibody assay that is to be used for the purpose of population seroprevalence studies. We compared 5,788 health care worker (HCW) serum samples by using two serological assays (Abbott SARS-CoV-2 anti-nucleocapsid immunoglobulin G (IgG) and Roche anti-SARS-CoV-2 anti-nucleocapsid total antibody) and a subset of samples (all Abbott assay positive or grayzone, $n = 485$) on Wantai SARS-CoV-2 anti-spike antibody enzyme-linked immunosorbent assay (ELISA). For 367 samples from HCW with a previous PCR-confirmed SARS-CoV-2 infection, we correlated the timing of infection with assay results. Overall, seroprevalence was 4.2% on Abbott and 9.5% on Roche. Of those with previously confirmed infection, 41% (150/367) and 95% (348/367) tested positive on Abbott and Roche, respectively. At 21 weeks (150 days) after confirmed infection, positivity on Abbott started to decline. Roche positivity was retained for the entire study period (33 weeks). Factors associated ($P \leq 0.050$) with Abbott seronegativity in those with previous PCR-confirmed infection included sex (odds ratio [OR], 0.30 male ; 95% confidence interval [CI], 0.15 to 0.60), symptom severity (OR 0.19 severe symptoms; 95% CI, 0.05 to 0.61), ethnicity (OR, 0.28 Asian ethnicity; 95% CI, 0.12 to 0.60), and time since PCR diagnosis (OR, 2.06 for infection 6 months previously; 95% CI, 1.01 to 4.30). Wantai detected all previously confirmed infections. In our

Citation Allen N, Brady M, Carrion Martin AI, Domegan L, Walsh C, Houlihan E, Kerr C, Doherty L, King J, Doheny M, Griffin D, Molloy M, Dunne J, Crowley V, Holmes P, Keogh E, Naughton S, Kelly M, O'Rourke F, Lynagh Y, Crowley B, de Gascun C, Holder P, Bergin C, Fleming C, Ni Riain U, Conlon N, on behalf of the PRECISE Study Steering Group. 2021. SARS-CoV-2 antibody testing in health care workers: a comparison of the clinical performance of three commercially available antibody assays. Microbiol Spectr 9:e00391-21. https://doi.org/10.1128/Spectrum.00391-21.

Editor Yun Young Go, City University of Hong Kong

Ad Hoc Peer Reviewer Kamoru ADEDOKUN, King Saud University

Address correspondence to Niamh Allen, nallen@stjames.ie, or Melissa Brady, melissa.brady1@hse.ie.

Comparison of SARS-CoV-2 antibody assays in healthcare workers; superior performance of anti-N panantibody assays over anti-N IgG assay @stjamesdublin @hpscireland

Received 26 May 2021
Accepted 18 August 2021
Published 29 September 2021

population, Roche detected antibodies up to at least 7 months after natural infection with SARS-CoV-2. This finding indicates that the Roche total antibody assay is better suited than Abbott IgG assay to population-based studies. Wantai demonstrated high sensitivity, but sample selection was biased. The relationship between serological response and functional immunity to SARS-CoV-2 infection needs to be delineated.

IMPORTANCE As the COVID-19 pandemic progresses, retained sensitivity over time is an important quality in an antibody assay that is to be used for the purpose of population seroprevalence studies. There is a relative paucity of published literature in this field to help guide public health specialists when planning seroprevalence studies. In this study, we compared results of 5,788 health care worker blood samples tested by using two assays (Roche and Elecsys, anti-nucleocapsid antibody) and by testing a subset on a third assay (Wantai enzyme-linked immunosorbent assay [ELISA] anti-spike antibody). We found significant differences in the performance of these assays, especially with distance in time from PCR-confirmed COVID-19 infection, and we feel these results may significantly impact the choice of assay for others conducting similar studies.

KEYWORDS Abbott Architect SARS-CoV-2 IgG, COVID-19 serological assays, Roche Elecsys SARS-CoV-2 panantibody, SARS-CoV-2 serological assay, SARS-CoV-2 seroprevalence, antibody assays SARS-CoV-2

Serological assays for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies have a significant role to play in the response to the COVID-19 pandemic. The detection of antibodies to SARS-CoV-2 is an excellent indicator of past SARS-CoV-2 infection (1) and therefore helps determine the proportion of a population that has been previously exposed or infected. Population seroprevalence studies can help account for asymptomatic cases and symptomatic individuals who may not present to health services for testing. As mass COVID-19 vaccination programs are rolled out globally, serological assays may have a role in assessing host vaccine response and predicting vaccine effectiveness (2). They may also potentially be used to inform individual risk of disease (3) with emerging evidence of up to 6 months of immunity after natural infection (4), although further research in this area of postinfection immunity is required.

Many studies have examined the timing of antibody (Ab) production and sensitivity of commercially available antibody assays in the early stages of infection with SARS-CoV-2 (5) (5). Few studies have evaluated and compared the longevity of assay sensitivity after PCR-confirmed infection for different assays on the same health care worker (HCW) population. As the pandemic progresses, retained sensitivity over time is an important quality in an antibody assay that is to be used for the purpose of population seroprevalence studies. A decline in SARS-CoV-2 antibody response to natural infection over time has been described (6, 7), and different antibodies may wane at different rates. Other studies have shown sustained antibody detection for up to 100 to 125 days (8, 9). Understanding the duration of antibody response over time is key to understanding the accuracy of epidemiological studies and informing public health pandemic response measures. The extent and duration of immunity and its relationship to antibody positivity are not yet fully understood; however, the use of serology at an individual level for the purpose of estimating immunity also requires detailed understanding of the timing and waning of antibody response in relation to PCR positivity. A UK study comparing five widely available commercial assays (including the Abbott Architect immunoglobulin G (IgG) assay and Roche Elecsys anti-SARS-CoV-2 assay) found them all to have a sensitivity and specificity of at least 98% at 30 days after symptom onset (10). Regarding the longevity of the antibody response, there are few published data, and results differ; a study comparing four different serological assays showed a decline in the performance of the Abbott assay after 60 days and a further decline after 80 days (11), whereas another study of the same assay showed a

mean of 137 days to loss of positive antibody (12). A study comparing the same assays as our study highlighted better performance of the Roche assay over the Abbott assay, although numbers were small (13). Further data comparing antibody assays for the detection of antibodies to SARS-CoV-2 are needed to guide the most appropriate use of certain commercially available assays in any given situation.

The primary purpose of the data collected was to estimate the seroprevalence of past SARS-CoV-2 infection in our HCW population; that analysis was carried separately as part of the PRECISE Study on Prevalence of Antibodies to SARS-CoV-2 in Irish Healthcare Workers (14). The data presented here are a secondary analysis as part of the PRECISE study, with the aim of comparing the prevalence of anti-SARS-CoV-2 antibodies in the same HCW population in two different assays targeting the anti-nucleocapsid protein, namely, Abbott Architect SARS-CoV-2 IgG assay and Roche Elecsys anti-SARS-CoV-2 immunoassay (15–17). We aimed to also compare antibody prevalence for a subset of samples on an assay targeting the anti-spike protein, namely, the Wantai SARS-CoV-2 antibody ELISA.

RESULTS

COVID-19 serology test results were available for 5,788 participating HCWs (comprising 64% of all staff in 2 Irish tertiary referral hospitals). The majority of participants were female (77%); the median age was 39 years (interquartile range [IQR], 30 to 49). The characteristics of participants tested by serology assay are shown in Appendix 1A and 1B.

SARS-CoV-2 seropositivity in relation to assay type: Abbott SARS-CoV-2 and Roche Anti-SARS-CoV-2. All 5,788 participants were tested using the Abbott SARS-CoV-2 IgG assay and 99.9% (n = 5,787) were tested using the Roche Anti-SARS-CoV-2 assay. A considerably lower proportion of participants had a positive antibody result on the Abbott assay (4.2%) than the proportion that had a positive antibody result on the Roche assay (9.5%) (Appendix 2A).

Assay concordance was moderate (k, 0.53; 95% confidence interval [CI], 0.48 to 0.57) (Table 1). McNemar's test for the difference in proportions indicated a systematic difference in the proportion of positive results between the two assays ($P < 0.001$). Twenty-four participants tested positive on Abbott but negative on Roche. Of the 24 participants, 3 also tested positive on the Wantai assay, suggesting possible false-negative results on Roche for these 3 participants. Of the remaining 21 participants, all tested negative on Wantai; none had a prior PCR-confirmed SARS-CoV-2 infection. An analysis was carried out in order to explore the association between participant characteristics and discordant results between the Abbott and Roche assays, and there was no significant association observed (data not presented here).

Abbott SARS-CoV-2 "Grayzone" results. Four percentage of participants (n = 221/5,787) had results in the Abbott SARS-CoV-2 IgG grayzone. Of those participants, 67% (n = 149/221) tested positive on the Roche assay. Among all of those with grayzone results, 42% (n = 93/221) were previously diagnosed with COVID-19 (SARS-CoV-2 positive by PCR). There were no participant characteristics that were significantly associated with having a result in the Abbott grayzone. In order to explore whether the numerical value within the grayzone sample to calibrator (S/C) index range could be used to assist in the interpretation of the grayzone, arbitrary cutoffs (low, medium, and

TABLE 1 Distribution of positive and negative results by serology assay

	Abbott SARS-CoV-2 IgG (n)				
Roche anti-SARS-CoV-2 result	Positive result	Negative or grayzone result	Total	Percentage agreement	κ^a Statistic (95% CI)
Positive	218	329	547	93.9	0.53 (0.48–0.57)
Negative	24	5,216	5,240		
Total	242	5545	5,787		

$^a\kappa$, Cohen's kappa.

TABLE 2 Distribution of positive and negative results by assay

Roche anti-SARS-CoV-2 result	Wantai SARS-CoV-2 Ab ELISA (*n*)			Percentage agreement	κ^a Statistic (95% CI)
	Positive result	Negative result	Total		
Positive	386	3	389	97.7	0.93 (0.88–0.97)
Negative	8	88	96		
Total	394	91	485		

[a]κ, Cohen's kappa.

high) were applied, and they were compared to the interpreted results on the Roche assay. There was no correlation observed, as a similar proportion of results within each of the arbitrary cutoffs was positive on the Roche assay (Appendix 2B).

SARS-CoV-2 seropositivity in relation to assay type: Roche Anti-SARS-CoV-2 and Wantai SARS-CoV-2 Ab ELISA. In total, 8.4% (*n* = 485/5,788) of participants were tested using the Wantai assay (of whom all were also tested using the Roche assay). Among these 485 participants, assay concordance was almost perfect (k, 0.93; 95% CI, 0.88 to 0.97) (Table 2). There was no evidence of differences in the proportion of positive results between the two assays ($P = 0.131$).

SARS-CoV-2 seropositivity over time (previously PCR-positive participants). A total of 367 participants were previously diagnosed COVID-19 positive by PCR. Among those participants, 41% (150/367) (95% CI, 35 to 46) tested positive on Abbott and 95% (348/367) (95% CI, 92 to 96) tested positive on Roche. There were 93 participants who were previously PCR positive who had a result in the Abbott grayzone. If the Abbott grayzone was included in positive results, Abbott positivity would increase to 66% (243/367). Of those participants diagnosed previously as COVID-19 positive by PCR, 71% (*n* = 259/367) were tested on Wantai, and all 259 had a positive result on the Wantai assay. Results for the Wantai assay are excluded from this section, as not all samples were tested on this platform.

The self-reported date of a previous positive PCR test was available for 365 (99%) participants. The interval between date of previous positive PCR test and date of serology test ranged from 12 to 231 days (2 to 33 weeks; 0 to 7 months). Serology test results by the number of weeks since a positive PCR test (including the breakdown for those who were symptomatic at the time of PCR testing) are shown in Table 3 and visually represented (by number of days) in Fig. 1 (visual representation excludes participants with grayzone results). We saw a decline in antibody positivity on the Abbott assay from week 21 (day 150) onward. The percent positivity by the number of months since a PCR test, including 95% CIs, is visually represented in Appendix 3A and 3B and shows a decline in antibody positivity on the Abbott assay in month 4.

The most common interval between positive PCR test and serology testing was 6 months (61%; *n* = 222). There were 210 participants who had a 6-month PCR-to-serology testing interval and who were symptomatic at the time of their PCR test; among them, positivity was 35% (95% CI, 29 to 41) on Abbott (*n* = 73) and was 93% (95% CI, 89 to 96) on Roche (*n* = 196).

Seventeen participants (4.6%) with a previously PCR-confirmed infection had a negative serology test result on both the Abbott and Roche assays and also tested negative on the Wantai assay. Of these 17 participants, 1 had a recent COVID-19 infection (24 days prior) and the remaining 16 had a distant infection (≥5 months prior to serology testing). Characteristics of the 17 participants are shown in Appendix 3C. Among the 17 participants, a lower proportion (88%; *n* = 15) had symptoms at the time of their PCR-positive test than the overall PCR-positive subgroup (98%; *n* = 358). A higher proportion participants were of white Irish background (94% versus 65% in the overall PCR-positive subgroup). Other characteristics did not differ considerably. An analysis was carried out in order to explore the association between participant characteristics and negative serology results; there was no significant association observed (data not presented here).

TABLE 3 Detection of SARS-CoV-2 antibodies by serology assay type with respect to time since positive PCR test[a]

	Previously PCR positive (n = 367)										Previously PCR positive and had symptoms at time of PCR test (n = 340)									
	Abbott SARS-CoV-2 IgG						Roche anti-SARS-CoV-2				Abbott SARS-CoV-2 IgG						Roche anti-SARS-CoV-2			
No. of wks	N	G	P	T	% Positive	% Positive (valid test)	N	P	T	% Positive	N	G	P	T	% Positive	% Positive (valid test)	N	P	T	% Positive
2	0	0	5	5	100	100	1	4	5	80.0	0	0	2	2	100	100	1	1	2	50.0
3	1	0	0	1	0.0	0.0	1	0	1	0.0	1	0	0	1	0.0	0.0	1	0	1	0.0
4	0	1	2	3	66.7	100	1	2	3	66.7	0	1	2	3	66.7	100	1	2	3	66.7
5	0	0	4	4	100	100	0	4	4	100	0	0	4	4	100	100	0	4	4	100
6	0	0	1	1	100	100	0	1	1	100	0	0	1	1	100	100	0	1	1	100
7	0	0	2	2	100	100	0	2	2	100	0	0	2	2	100	100	0	2	2	100
9	0	0	1	1	100	100	0	1	1	100	0	0	1	1	100	100	0	1	1	100
10	0	0	2	2	100	100	0	2	2	100	0	0	2	2	100	100	0	2	2	100
11	0	0	1	1	100	100	0	1	1	100	0	0	0	0	100	100	0	0	0	100
17	0	0	1	1	100	100	0	1	1	100	0	0	1	1	100	100	0	1	1	100
19	0	0	2	2	100	100	0	2	2	100	0	0	2	2	100	100	0	2	2	100
20	0	0	1	1	100	100	0	1	1	100	0	0	1	1	100	100	0	1	1	100
21	0	1	5	6	83.3	100	0	6	6	100	0	1	5	6	83.3	100	0	6	6	100
22	0	4	7	11	63.6	100	0	11	11	100	0	3	6	9	66.7	100	0	9	9	100
23	3	1	7	11	63.6	70.0	1	10	11	90.9	2	1	7	10	70.0	77.8	0	10	10	100
24	6	5	6	17	35.3	50.0	0	17	17	100	5	5	5	15	33.3	50.0	0	15	15	100
25	9	12	12	33	36.4	57.1	0	33	33	100	9	11	12	32	37.5	57.1	0	32	32	100
26	7	10	7	24	29.2	50.0	1	23	24	95.8	7	9	7	23	30.4	50.0	1	22	23	95.7
27	18	7	23	48	47.9	56.1	2	46	48	95.8	16	7	21	44	47.7	56.8	2	42	44	95.5
28	20	9	18	47	38.3	47.4	2	45	47	95.7	19	8	17	44	38.6	47.2	1	43	44	97.7
29	20	17	24	61	39.3	54.5	6	55	61	90.2	19	16	24	59	40.7	55.8	6	53	59	89.8
30	31	18	9	58	15.5	22.5	4	54	58	93.1	29	18	8	55	14.5	21.6	4	51	55	92.7
31	8	7	6	21	28.6	42.9	0	21	21	100	6	7	6	19	31.6	50.0	0	19	19	100
32	0	1	2	3	66.7	100	0	3	3	100	0	1	2	3	66.7	100	0	3	3	100
33	1	0	0	1	0.0	0.0	0	1	1	100	1	0	0	1	0.0	0.0	0	1	1	100
Total	124	93	150	367	40.9	54.7	19	348	367	94.8	114	88	138	340	40.6	54.8	17	323	340	95.0

[a]Excludes two participants for which time since positive PCR test was unknown. Values are serology test results, as follows: N, number negative; G, number grayzone; P, number positive; T, total tested; % positive valid test excluding grayzone results.

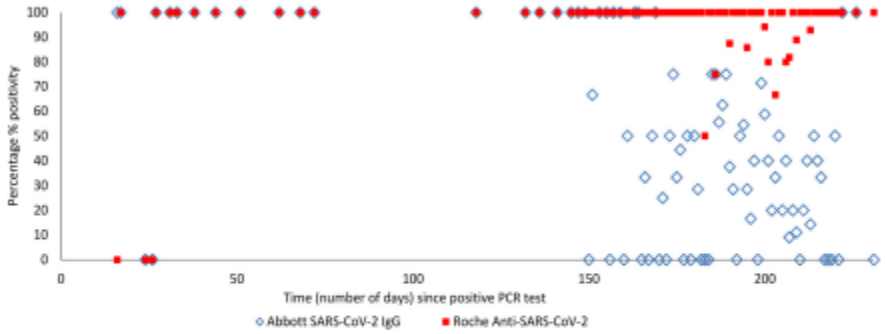


FIG 1 Percentage of participants with detected SARS-CoV-2 antibodies by serology assay with respect to time (number of days) since a positive PCR test, among participants who had previous PCR-confirmed COVID-19 infection and had symptoms at the time of their PCR test ($n = 340$).

In order to explore whether the Roche quantitative cutoff index (COI) or the Abbott S/C index was close to the positive threshold for these 17 participants, arbitrary cutoffs (low, medium, and high negative) were applied to the results of both assays. For the Roche assay, three participants had results that were close to the positive result threshold (i.e., in the high negative range; COI, 0.6 to 0.9), six had results in the medium negative range (COI, 0.3 to 0.6), and eight had results in the low negative range (COI, 0 to 0.3). For the Abbott assay, all participants had results in either the medium negative range ($n = 3$) or the low negative range ($n = 14$).

Abbott SARS-CoV-2 seronegativity in relation to participant characteristics. The characteristics of participants with previous PCR-confirmed SARS-CoV-2 infection ($n = 367$) and their serology test results by Abbott SARS-CoV-2 are shown in Appendix 3D and 3E. In total, 45% ($n = 124$) (34% including those who had grayzone results in the total number tested) of participants with previous PCR-confirmed SARS-CoV-2 infection did not have detectable antibodies on the Abbott assay. Univariate and multivariable analyses were carried to out to explore the association between participant characteristics and the negative Abbott test result. Table 4 presents the results of the analysis. Factors associated with Abbott seronegativity in those with a previous PCR-confirmed infection varied by sex (male adjusted odds ratio [aOR], 0.30; 95% CI, 0.15 to 0.60; $P < 0.001$), symptom severity (severe symptoms requiring hospitalization; aOR, 0.19; 95% CI, 0.05 to 0.61; $P = 0.008$), ethnicity (Asian ethnicity; aOR, 0.28; 95% CI, 0.12 to 0.60; $P = 0.001$), and time since PCR diagnosis (increase from 5- to 6-month interval; aOR, 2.06; 95% CI, 1.01 to 4.30; $P = 0.050$).

To broaden the analysis, a multivariable logistic regression was repeated, including participants that had grayzone results. The results of this analysis were similar to the results of the initial analysis and are presented in Appendix 4. A separate analysis was carried out to explore the association between participant characteristics and Abbott grayzone results; there was no significant association observed (data not presented).

DISCUSSION

Seropositivity in relation to assay type: Abbott versus Roche—all participants. In agreement with recently published data (11), a considerably higher proportion of participants (more than double) had detectable antibodies on the Roche assay than on the Abbott assay. In terms of assay performance, according to the manufacturer's instructions, both assays performed with high sensitivity and high specificity. Applying the lower bounds of the confidence interval for specificity of the Abbott assay, a maximum false-negative rate of 4.2% ($n = 15$) could be expected; therefore, 109 out of 124 negative test results among those previously diagnosed with COVID-19 (positive by

TABLE 4 Factors associated with Abbott SARS-CoV-2 seronegativity, among participants with previous PCR-confirmed SARS-CoV-2 infection[a]

Parameter	All participants (*n*)	Negative result (*n*)	Negative %	Unadjusted			Adjusted		
				OR	95% CI	*P* value[b]	OR	95% CI	*P* value[b]
Total	274	124	45.3						
Age group (in yrs)									
18–29	79	41	51.9	*[c]			*		
30–39	79	43	54.4	1.11	0.59–2.07	0.750	0.99	0.46–2.15	0.982
40–49	59	17	28.8	**0.38**	**0.18–0.76**	**0.007**	**0.36**	**0.15–0.82**	**0.017**
50–59	45	19	42.2	0.68	0.32–1.41	0.301	0.70	0.28–1.71	0.429
60+	12	4	33.3	0.46	0.12–1.60	0.238	0.25	0.05–1.04	0.065
Sex									
Female	201	102	50.7	*			*		
Male	73	22	30.1	**0.42**	**0.25–0.74**	**0.003**	**0.30**	**0.15–0.60**	**<0.001**
Ethnicity									
Irish (white)	173	94	54.3	*			*		
Any other white background	35	12	34.3	0.44	0.20–0.92	0.033	0.49	0.2–1.19	0.118
Any Asian background	56	14	25.0	**0.28**	**0.14–0.54**	**<0.001**	**0.28**	**0.12–0.60**	**0.001**
Any African/black background	7	2	28.6	0.34	0.04–1.61	0.200	0.47	0.05–3.33	0.449
Other	3	2	66.7	1.68	0.15–36.6	0.067	2.33	0.14–70.6	0.566
Close contact with a case of COVID-19[d]									
Yes	155	65	41.9	0.71	0.44–1.15	0.178	0.63	0.34–1.16	0.137
No	117	59	50.4	*			*		
Main type of patient contact[e]									
No patient contact	46	24	52.2	*			*		
Daily contact non-COVID-19 patients	162	74	45.7	0.77	0.39–1.49	0.437	0.79	0.34–1.80	0.577
Daily contact known or suspected COVID-19 patients	66	26	39.4	0.60	0.27–1.27	0.182	0.83	0.32–2.14	0.694
Severity of symptoms[d]									
No symptoms	6	3	50.0	0.82	0.15–4.61	0.816	0.86	0.12–6.29	0.876
Minor symptoms	113	62	54.9	*			*		
Significant symptoms	130	55	42.3	**0.60**	**0.36–1.00**	**0.051**	**0.50**	**0.27–0.92**	**0.028**
Severe symptoms (hospitalized)	23	4	17.4	**0.17**	**0.04–0.49**	**0.003**	**0.19**	**0.05–0.61**	**0.008**
HCW role									
Administration	18	9	50.0	*			Not included in the model		
Allied health care	34	19	55.9	1.27	0.40–4.03	0.686			
General support	10	5	50.0	1.00	0.21–4.81	1.00			
Health care assistant	24	5	20.8	0.26	0.06–0.98	0.053			
Medical/dental	62	29	46.8	0.88	0.30–2.54	0.809			
Nursing/midwifery	124	55	44.4	0.80	0.29–2.17	0.653			
Other	2	2	100.0	NA[f]	NA	NA			
No. of mo since PCR-positive test[d]									
Less than 1	7	1	14.3	0.25	0.01–1.57	0.21	0.11	0.01–0.83	0.061
1	8	0	0.0	NA	NA	NA	NA	NA	NA
2	4	0	0.0	NA	NA	NA	NA	NA	NA
3	1	0	0.0	NA	NA	NA	NA	NA	NA
4	10	0	0.0	NA	NA	NA	NA	NA	NA
5	57	23	40.4	*			*		
6	168	91	54.2	**1.75**	**0.95–3.25**	**0.073**	**2.06**	**1.01–4.30**	**0.050**
7	17	9	52.9	1.66	0.57–5.05	0.360	2.67	0.74–10.2	0.139

[a]*n* = 274 participants with valid results.
[b]*P* values were calculated using the chi-square test, results for significant associations are highlighted in bold.
[c]*, reference category.
[d]Excludes 2 unknowns.
[e]Participants were asked which type of patient contact describes most of their current work (excludes 5 unknowns).
[f]NA, insufficient number of participants in this category.

PCR) could not be explained by expected test performance; it is possible that SARS-CoV-2 IgG was absent among these individuals. Applying the lower bounds of the confidence interval for specificity of the Roche assay, a maximum false-negative rate of 0.3% (n = 1) could be expected; therefore, 18 out of 19 negative test results among those previously diagnosed with COVID-19 could not be explained by expected test performance. It is possible that some of these individuals did not mount a SARS-CoV-2 antibody response to their infection; however, in the published literature, the majority of patients have been shown to develop antibodies following natural infection (18–20). Therefore, it is likely that the main reason for lack of antibodies in this subset of participants was due to a waning antibody response that was not picked up on testing, particularly on the Abbott assay. Studies have shown that anti-nucleocapsid IgG antibodies may be less likely to develop than IgM antibodies; Zhao et al. showed that at day 15 postinfection, 100% had detected total Ab, 94.3% had IgM, and only 79.8% had IgG Ab (18). Peterson et al. and Kaufman et al. showed that IgG antibodies were not detected for between 6.3% and 9% of people in the first 2 weeks after PCR confirmation of infection (20, 21) and that this proportion rose to 14% by 99 to 121 days (22).

Lumley at al. demonstrated stable anti-spike IgG antibodies but waning anti-nucleocapsid IgG antibodies over time among HCWs in the United Kingdom health care service (12), as did Pelleau et al. (23). This result may in part explain the Abbott IgG assay's relative performance for detecting past infection, compared with the Roche total antibody assay. The total antibody approach used in the Roche Elecsys system results in improved and sustained sensitivity suitable for population seroprevalence studies.

Twenty-four participants had detectable antibodies on the Abbott assay but not on the Roche assay. Of those participants, three had detectable antibodies on the Wantai assay (one of whom had recent PCR-confirmed infection) and were presumably false negatives on Roche, possibly reflecting differences in test method and target between the Wantai and Roche assays.

Does the Abbott grayzone add anything? In October 2020, Abbott updated their guidance on their Architect SARS-CoV-2 IgG assay (Abbott Diagnostics Product Information Letter PI1060-2020) to include an optional editable "grayzone" with a S/C index range of 0.5 to 1.39 which they advise "must be interpreted by the clinician in the context of relevant clinical and laboratory information on the patient" (16). The grayzone accounts for a large proportion of the disagreement between the assays. The majority of grayzones were positive by Roche (67%) and Wantai (70%). However, interpretation of this Abbott grayzone result in a clinical setting would not be straightforward, and confirmatory testing would still be needed. There was no obvious correlation between increasing or decreasing Abbott grayzone S/C indices and the interpreted results from other assays, so while the grayzone may add value for individual serology testing (by indicating a need for additional testing), we feel that it does not add value in the setting of population-based serological studies. In an epidemiological study setting such as this, inclusion of all grayzone results as presumptive positives would lead to a significant overestimation of seroprevalence. Studies such as ours could be used to estimate the proportion of grayzone results likely to be positive on other testing platforms; however, the use of alternative assays with prolonged sensitivity over time would be superior if the primary purpose is to estimate infection ever. It is notable that while studies have shown good protection against reinfection over at least a 6-month period following PCR-confirmed COVID-19 infection (4), to the best of our knowledge, no studies have yet compared antibody assays in terms of functional immunity.

Seropositivity in relation to assay type—Roche versus Wantai assay. We found almost perfect agreement between the Roche and Wantai assays. A study comparing eight assays found these two assays to provide the highest sensitivities at 98% and 95%, respectively (24), which, in agreement with our findings, suggest that total antibody assays are better positioned for population-based serological testing. However, in our study, the assay concordance should be interpreted with caution, as the selection of participants for additional testing on Wantai ELISA was heavily biased; only participants who had a positive or grayzone Abbott SARS-CoV-2 IgG result were selected for testing by the Wantai assay. The small degree of discordance between these two

total antibody assays could be due to a number of reasons, including the protein targeted (anti-N versus anti-S) and the different expected performances according to manufacturer guidelines (25).

Seropositivity over time (previously PCR-positive participants). Of the subset of participants who had previous PCR-confirmed COVID-19 infection, the Roche assay performed better in terms of identifying these participants and 95% tested positive. Only one-half of these past infections were accurately identified by the Abbott assay. Harley et al. showed a much higher level of agreement between these two assays, although on a smaller number of samples than were tested in our study and at an earlier time point after PCR-confirmation of infection (26). Therefore, antibody waning may not yet have occurred to the degree that it had occurred in our study All previously PCR-positive participants in our study had detectable antibodies on the Wantai ELISA, but this subpopulation examination and its inherent selection bias limit strong conclusions from these data.

The majority of participants had a 6-month interval between confirmed infection and antibody testing in our study, which correlated with the time period between the peak of the first wave of the pandemic in Ireland and the antibody testing in our study. Although detection was slightly lower on both assays than the overall detection of previous infections occurring at any time, the Roche assay still performed significantly better than Abbott using the 6-month time frame, identifying 93% versus 46% of infections that occurred 6 months prior.

The decay in seropositivity on Abbott in our cohort started at 21 weeks (150 days) after confirmed infection, but the small numbers of infection per week prior to this time should be noted. This decay did not change with removal of participants who had no symptoms at the time of infection. A study comparing four different serological assays, including the Abbott and Roche assays, showed a decline in the performance of the Abbott assay after 60 days, whereas antibodies were still detected on the Roche assay after 80 days (11). In contrast, another study of the Abbott assay showed a mean of 137 days to the loss of positive antibodies (12). Kumar et al. showed a much shorter duration of antibody detection using the Roche assay after confirmed SARS-CoV-2 infection in a small cohort of Indian HCWs; seropositivity halved by day 30 to 42 and fell to 0 after 50 days (27), which may suggest an impact of host factors, such as ethnicity, on antibody production and waning. In a large US study, Kaufman et al. showed a decline in IgG antibodies (including on Abbott) to 74% by 2 months after confirmed infection (22). Due to the small numbers of infection per week prior to the 21-week time point in our study, it is possible that this decline in Abbott IgG positivity started at an earlier time point that was not picked up in our study. While we demonstrate good performance of the Roche anti-nucleocapsid assay at extended time points, performance of assays at time points even more distant from the infection remains obscure.

In a multivariable analysis, the risk of a negative result on the Abbott platform despite previous PCR-confirmed COVID-19 infection increased with the interval between infection and antibody testing, in keeping with antibody waning. Those who had moderate or severe symptoms were more likely to have retained IgG than those who had only mild symptoms. This result was consistent with the findings of other researchers who found that IgG was better sustained in persons reporting significant symptoms than those who had mild or no symptoms (24, 26, 27). Participants of male sex and Asian ethnicity were also more likely to have sustained Abbott assay-detected IgG. Kaufman et al. showed age and male sex to be associated with the probability of persistent IgG serology (22), as did a large Cochrane review conducted in April 2020 (28). Furthermore, Lumley at al. demonstrated that increasing age, Asian ethnicity, and prior self-reported symptoms were independently associated with higher maximum anti-nucleocapsid IgG levels (12). This result is in keeping with our findings on sex, ethnicity, and prior self-reported symptoms; however, we did not find any statistically significant correlation with age. The reason for this sustained IgG response in participants of male

sex is not yet clear but may be related to higher viral load, other indices of severity, or other unknown biological factors not included in this study.

Participants who had a 6-month PCR-to-serology testing interval were twice as likely to be IgG seronegative (Abbott) than participants who had a 5-month testing interval. Those who had a 7-month testing interval were also more likely to be IgG seronegative than those who had a 5-month testing interval, but results were not significant. Our findings are consistent with the findings of other researchers who have demonstrated a decline in IgG seropositivity over time, by using the Abbott and other IgG antibody assays (6, 23). Further studies may provide a better understanding of seropositivity and seroreversion over time, relating to specific assays or the type of immunoglobulin measured.

We did not have enough participants with very recent infection to assess the ability of each assay to detect early infection; however, of the 8 infections that occurred within 4 weeks of antibody testing, 8/9 were correctly identified by the Abbott assay and 6/9 were identified by the Roche assay. Higher numbers would be needed to compare these assays specifically in the early stages of infection.

Almost 5% of infections were not identified by either platform. The majority were distant infections (≥6 months ago), and therefore, waning immunity may explain the seronegativity. One was a recent infection (PCR positive 24 days prior to serological testing) in a participant who had severe symptoms; this infection may have been too recent to have detectable antibodies on either assay; however, four other participants with only minor symptoms and even more recent infections (PCR positive 12 to 17 days prior to serology) had antibodies detected on both Abbott and Roche assays. Overall, fewer of these participants with previous confirmed infection and negative serology on both platforms were symptomatic at the time of their infection; other studies have shown those without symptoms to be both less likely to develop antibodies and less likely to have persistent antibodies 8 weeks postinfection (26). There were no other participant characteristics that were significantly associated with negative serology, nor were these participants quantitative results (Abbott S/C and Roche COI index) close to the positive result threshold for either assay. Both assays performed below their expected sensitivity based on manufacturer guidelines, likely due to the time interval between infection and antibody testing. This shortcoming should be considered in serological studies, as well as when using antibody testing in the setting of clinical care. As the pandemic progresses, further studies may highlight the sensitivity of different assays and/or different antigenic targets with more accuracy in relation to the timing of testing.

Limitations. Our study has several limitations. First, as the main PRECISE seroprevalence study was not designed with the primary objective of comparing the serological assays, we did not provide a comparable assessment of specificity by testing of any prepandemic negative-control samples. Second, information on COVID-19 symptoms and PCR test results were self-reported and thus could be biased. The dates of PCR-confirmed infection (also self-reported) may be inaccurate, and participants may have been reinfected at a later date but unaware of it; given the timing of this study at 8 months after the first COVID-19 cases in Ireland and the evidence of 9 months of protection from reinfection following natural infection, it is unlikely that many, if any, participants in the study had been reinfected (29). Furthermore, the PCR cycle threshold (C_T) value which would have been a valuable addition to this study was not available. Other variables which would have been valuable to this study but were not available include participant comorbidities and host determinants of immune response; there may be individual pathophysiological factors that influence the immune response differently depending on the antigenic target. A small sample size for the 0- to 4-month PCR-to-serology test interval prevented a meaningful analysis of seropositivity and seronegativity at the early stages postinfection.

Our study focused on assays that have the SARS CoV-2 nucleocapsid as an antigenic target with only a subpopulation assessed using a spike antibody assay. Given emerging evidence of differences in antibody decay related to the antigenic target, a more complete

assessment would have been desirable. Such approaches, including parallel spike and nucleocapsid assessments, may become more relevant in the era of SARS CoV-2 vaccination. In addition, we did not address the neutralizing capacity of the antibodies measured, and therefore, conclusions about the functional consequences of the presence of such antibodies are limited.

Conclusion and recommendations. The Roche total antibody assay performed significantly better at identifying those who had ever had a confirmed COVID-19 infection than the Abbott IgG assay, although both assays were less sensitive than the manufacturer guidelines. Our study findings suggest that, of these two assays directed at anti-nucleocapsid antibodies, Roche is better suited to future population-based serological studies due to a maintained detection of total antibodies up to at least 7 months after natural infection with SARS-CoV-2. While maximum sensitivity is achieved using multiple testing platforms, this is not always feasible or cost-effective, and the overall seroprevalence results of our study would have been unchanged if only the Roche assay was used. While we demonstrate good performance of the Roche anti-nucleocapsid assay at extended time points, results of the comparison with other antigenic targets at time points even more distant from the infection remain obscure. The anti-spike assay (Wantai) performed very well on the subset of samples analyzed, but further studies are needed to show if it maintains its sensitivity compared with Roche on an unbiased selection of samples. The performance of both total antibody assays (Roche and Wantai) points toward the superiority of total antibody assays over IgG assays for serological studies.

The risk of a negative antibody result on the Abbott assay despite a previous PCR-confirmed SARS-CoV-2 infection increased with time since infection; the decline was noted at 21 weeks/150 days after a confirmed SARS-CoV-2 infection. Those participants of male sex and Asian ethnicity, and those with moderate to severe symptoms were more likely to retain IgG detection on the Abbott assay. The Abbott grayzone did not add anything that would eliminate the need for additional testing on an alternative assay. From our limited numbers of recent infections, it may be possible that the Abbott assay identified early infection quicker than the Roche assay, but our numbers were too small to confirm this hypothesis.

Our study adds to the growing literature on serological assays for population-based studies. Further data comparing SARS-CoV-2 antibody assays are needed to guide the most appropriate use of certain assays in any given situation, especially where the use of dual or multiple assays is not feasible or affordable. With the recent introduction of widespread vaccination, it is yet to be determined if measuring antibody response post vaccination is meaningful and cost-effective and, if so, which assays are superior. Further studies are also needed to delineate the relationship between serological response and functional immunity to SARS-CoV-2 infection, both following vaccination and natural infection. Assays with a prolonged sensitivity are likely to be more valuable as the pandemic continues, in particular anti-nucleocapsid antibody assays that will differentiate past natural infection from vaccine-induced immunity.

MATERIALS AND METHODS

Study design. This was a cross-sectional study of the seroprevalence of circulating antibodies to SARS-CoV-2 in hospital HCW, performed in October 2020 (30). All staff members of two Irish tertiary referral hospitals were invited to participate in an online self-administered consent process and online questionnaire, followed by blood sampling for SARS-CoV-2 antibody testing. These participants included both patient-facing and nonpatient facing staff members. Information collected in the questionnaire included demographic information and information regarding previous COVID-19 symptoms, testing, and diagnosis. Full details of the study design, study locations, recruitment, and study participants can be found in the published PRECISE study report (30).

Ethical approval. Ethical approval was obtained from the National Research Ethics Committee for COVID-19 in Ireland (20-NREC-COV-101).

Laboratory assays. All samples were tested by two assays, namely, Abbott SARS-CoV-2 measuring IgG (referred to here as Abbott) and Roche Elecsys Anti-SARS-CoV-2 measuring total antibodies (referred to here as Roche) (15–17). The Abbott assay is a chemiluminescent microparticle immunoassay that detects IgG antibodies to the nucleocapsid protein of SARS-CoV-2. The Roche assay is an electrochemiluminescent immunoassay that detects antibodies (including IgG), also to the nucleocapsid protein. Assay

TABLE 5 Summary of assay performance according to manufacturer specifications

Assay	Sensitivity (% [95% CI])	Specificity (% [95% CI])
Abbott SARS-CoV-2 IgG	100 (95.8–100)	99.6 (99.0–99.9)
Roche Anti-SARS-CoV-2	100 (88.3–100)	99.8 (99.7–99.9)
Wantai SARS-CoV-2 Antibody ELISA	96.7 (83.3–99.4)	97.5 (91.3–99.3)

results were interpreted using the manufacturers' recommended assay specific thresholds. Assay threshold of ≥1.4 (sample to calibrator [S/C] index) for Abbott and ≥1.0 (cutoff index [COI]) for Roche were determined to be reactive and interpreted as antibody positive. The Abbott SARS-CoV-2 IgG grayzone is an additional assay threshold band for potential positivity, suggested by the manufacturer to increase assay sensitivity (Abbott Diagnostics Product Information Letter PI1060-2020) (16). The interpretation of Abbott S/C indices within this study is as follows: negative, <0.5; grayzone, 0.5 to <1.4; and positive, ≥1.4. All samples with an Abbott result of positive or grayzone were tested on a third assay in the National Virus Reference Laboratory (NVRL) using the Wantai SARS-CoV-2 antibody ELISA (referred to here as Wantai), distributed by Fortress Diagnostics. Wantai is an enzyme-linked immunosorbent assay (ELISA) for qualitative detection of total antibodies (including IgG and IgM) to the spike protein of SARS-CoV-2.

In terms of assay performance, according to the manufacturer's specifications, all three assays perform with high sensitivity and high specificity (see Table 5) (25).

Statistical methods. Assay concordance was assessed using Cohen's kappa statistic and McNemar's test for difference in proportions. Cohen's kappa coefficient (κ) measures the level of agreement between assays, taking into account the possibility of the agreement occurring by chance. The κ statistic varies from 0 to 1, where 0 = agreement equivalent to chance, 0.1 to 0.20 = slight agreement, 0.21 to 0.40 = fair agreement, 0.41 to 0.60 = moderate agreement, 0.61 to 0.80 = substantial agreement, 0.81 to 0.99 = near perfect agreement, and 1 = perfect agreement. For analysis of assay concordance, results must be classified into the same number of mutually exclusive categories; therefore, Abbott negative and grayzone results were grouped. Confidence intervals for the proportion of participants that were seropositive were computed. Multivariable logistic regression was carried out to assess risk factors for the absence of SARS-CoV-2 IgG antibodies by controlling for age, sex, ethnicity/background, type of patient contact, severity of symptoms, and number of months since a PCR positive test. Forward stepwise selection was used, and the Akaike information criterion (AIC) was used to evaluate model efficiency; HCW role was excluded in the final model. We used R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria) and OpenEpi software version 3.01 (Wilson Score) (31).

Data availability. The original data set for this research may identify individuals and therefore is not being routinely deposited in a data repository. However, the data set can be made available upon reasonable request to the principal investigator.

APPENDIX

Appendix 1A Characteristics of participants tested, by serology assay

	Participants tested by:							
	Abbott SARS-CoV-2 IgG		Roche anti-SARS-CoV-2		Wantai SARS-CoV-2 antibody ELISA		Total participants	
Parameter	*n*	%	*n*	%	*n*	%	*n*	%
Total participants tested	5,788		5,787		485		5,788	
Age group (yrs)								
18–29	1,350	23.3	1,350	23.3	139	28.7	1,350	23.3
30–39	1,617	27.9	1,617	27.9	139	28.7	1,617	27.9
40–49	1,516	26.2	1,515	26.2	111	22.9	1,516	26.2
50–59	1,001	17.3	1,001	17.3	68	14.0	1,001	17.3
60+	304	5.3	304	5.3	28	5.8	304	5.3
Sex								
Female	4,478	77.4	4,478	77.4	349	72.0	4,478	77.4
Male	1,309	22.6	1,308	22.6	136	28.0	1,309	22.6
Unknown	1	0.0	1	0.0	0	0.0	1	0.0
Ethnicity								
Irish (white)	4,444	76.8	4,444	76.8	313	64.5	4,444	76.8
Any other white background	552	9.5	551	9.5	54	11.1	552	9.5
Any Asian background	577	10.0	577	10.0	101	20.8	577	10.0
Any African or black background	113	2.0	113	2.0	13	2.7	113	2.0
Other	101	1.7	101	1.7	4	0.8	101	1.7
Unknown	1	0.0	1	0.0	0	0	1	0.0
HCW role								
Nursing/midwifery	2,064	35.7	2,064	35.7	230	47.4	2,064	35.7
Allied health	1,091	18.8	1,091	18.9	57	11.8	1,091	18.8
Medical/dental	983	17.0	982	17.0	93	19.2	983	17.0
Admin	803	13.9	803	13.9	33	6.8	803	13.9
General support	434	7.5	434	7.5	21	4.3	434	7.5
Health care assistant	286	4.9	286	4.9	45	9.3	286	4.9
Other	127	2.2	127	2.2	6	1.2	127	2.2

Appendix 1B COVID-19 related characteristics of participants tested, by serology assay

	Abbott SARS-CoV-2 IgG		Roche Anti-SARS-CoV-2		Wantai SARS-CoV-2 Antibody ELISA		Total participants	
Parameter	*n*	%	*n*	%	*n*	%	*n*	%
Total participants tested	5,788		5,787		485		5,788	
Close contact with a case of COVID-19								
Yes	1,705	29.5	1,704	29.4	258	53.2	1,705	29.5
No	4,071	70.3	4,071	70.3	225	46.4	4,071	70.3
Unknown	12	0.2	12	0.2	2	0.4	12	0.2
Main type of patient contact[a]								
Daily contact with known/suspected COVID-19 patients	903	15.6	902	15.6	109	22.5	903	15.6
Daily contact with patients without COVID-19	3,245	56.1	3,245	56.1	299	61.6	3,245	56.1
No patient contact	1,635	28.2	1,635	28.3	77	15.9	1,635	28.2
Unknown	5	0.1	5	0.1	0	0.0	5	0.1
Previous COVID-19 symptoms (ever)								
No symptoms	2,869	49.6	2,868	49.6	102	21.0	2,869	49.6
Had symptoms	2911	50.3	2,911	50.3	381	78.6	2,911	50.3
Unknown	8	0.1	8	0.1	2	0.4	8	0.1
Severity of symptoms								
Minor symptoms	2,159	74.2	2,159	74.2	193	50.7	2,159	74.2
Significant symptoms	701	24.1	701	24.1	161	42.3	701	24.1
Severe symptoms (hospitalized)	51	1.8	51	1.8	27	7.1	51	1.8
Previous COVID-19 PCR test								
Yes	2,779	48.0	2,778	48.0	380	78.4	2,779	48.0
No	3,003	51.9	3,003	51.9	105	21.6	3,003	51.9
Unknown	6	0.1	6	0.1	0	0.0	6	0.1
Previous positive COVID-19 PCR test								
Yes	367	6.3	367	6.3	259	53.4	367	6.3
No	5,415	93.6	5,414	93.6	226	46.6	5,415	93.6
Unknown	6	0.1	6	0.1	0	0.0	6	0.1

[a]Participants were asked which one describes most of their current work.

Appendix 2A Serology result by assay[a,c,d]

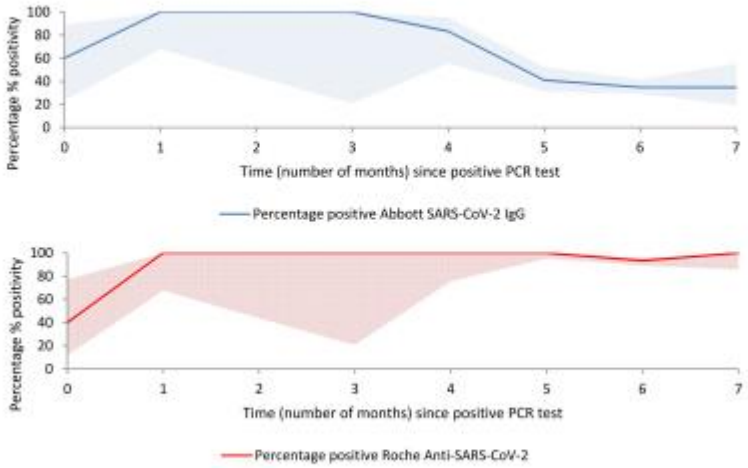
Assay	Negative	Grayzone	Positive	Total[b]	% Positive	Total valid[c]	% Positive (where valid)
Abbott SARS-CoV-2 IgG	5,325	221	242	5,788	4.2	5,567	4.3
Roche Anti-SARS-CoV-2	5,240		547	5,787	9.5	5,787	9.5

[a]Values are no. of participants unless otherwise stated.
[b]Total, total tested.
[c]Total valid, total no. tested excluding grayzone test result.
[d]This table excludes the Wantai assay due to sampling bias.

Appendix 2B Comparison of Abbott SARS-CoV-2 IgG S/C index and Roche anti-SARS-CoV-2 interpreted result, among participants with grayzone results[a]

	Result by assay:			
	Abbott SARS-CoV-2	Roche anti-SARS-CoV-2		
Arbitrary cutoff	Grayzone (*n*)	Negative (*n*)	Positive (*n*)	Positivity (%)
Low grayzone (S/C 0.5–0.8)	115	39	76	66.1
Medium grayzone (S/C 0.8–1.1)	62	18	44	71.0
High grayzone (S/C 1.1–1.4)	44	15	29	65.9
Total	221	72	149	67.4

[a]n = 221.



Appendix 3A and 3B Percentage of participants with detected SARS-CoV-2 antibodies by serology assay with respect to time (number of months) since a positive PCR test, among participants who had previous PCR-confirmed COVID-19 infection and had symptoms at the time of their PCR test ($n = 340$). Shaded area shows the 95% confidence interval.

Appendix 3C Characteristics of participants who had a previously PCR-confirmed infection and were negative by serology testing

Parameter	*n*	%
Total	17	
Age group		
18–29	5	29.4
30–39	4	23.5
40–49	4	23.5
50–59	4	23.5
60+	0	0.0
Sex		
Female	14	82.4
Male	3	17.6
Ethnicity		
Irish (white)	16	94.1
Any other white background	1	5.9
HCW role		
Nursing/midwifery	7	41.2
Medical/dental	4	23.5
Admin	1	5.9
Allied health	2	11.8
Health care assistant	2	11.8
General support	1	5.9
Close contact with a case of COVID-19		
Yes	7	41.2
No	10	58.8
Main type of contact with COVID-19 patients		
Daily contact with known/suspected COVID-19 patients	3	17.6
Daily contact with patients without COVID-19	11	64.7

(Continued on next page)

Appendix 3C (Continued)

Parameter	*n*	%
No patient contact	3	17.6
Previous COVID-19 symptoms		
No symptoms	2	11.8
Had symptoms	15	88.2
Severity of symptoms		
Minor symptoms	7	46.7
Significant symptoms	8	53.3
Severe symptoms (hospitalized)	0	0.0
No. of mo since positive PCR test		
Less than 1	1	5.9
5	1	5.9
6	15	88.2

Appendix 3D Characteristics of participants who had a previously PCR-confirmed infection and their test results on Abbott SARS-CoV-2 IgG

			Test result					
	All participants		Grayzone		Positive		Negative	
Parameter	*n*	%	*n*	%	*n*	%	*n*	%
Total	367		93		150		124	
Age group								
18–29	111	30.2	32	34.4	38	25.3	41	33.1
30–39	108	29.4	29	31.2	36	24.0	43	34.7
40–49	79	21.5	20	21.5	42	28.0	17	13.7
50–59	50	13.6	5	5.4	26	17.3	19	15.3
60+	19	5.2	7	7.5	8	5.3	4	3.2
Sex								
Female	269	73.3	68	73.1	99	66.0	102	82.3
Male	98	26.7	25	26.9	51	34.0	22	17.7
Ethnicity								
Irish (white)	237	64.6	64	68.8	79	52.7	94	75.8
Any other white background	44	12.0	9	9.7	23	15.3	12	9.7
Any Asian background	72	19.6	16	17.2	42	28.0	14	11.3
Any African or black background	10	2.7	3	3.2	5	3.3	2	1.6
Other	4	1.1	1	1.1	1	0.7	2	1.6
HCW role								
Nursing/midwifery	181	49.3	57	61.3	69	46.0	55	44.4
Allied health	42	11.4	8	8.6	15	10.0	19	15.3
Medical/dental	73	19.9	11	11.8	33	22.0	29	23.4
Administration	24	6.5	6	6.5	9	6.0	9	7.3
General support	12	3.3	2	2.2	5	3.3	5	4.0
Health care assistant	32	8.7	8	8.6	19	12.7	5	4.0
Other	3	0.8	1	1.1	0	0.0	2	1.6

Appendix 3E COVID-19 related characteristics of participants who had a previously PCR-confirmed infection and their test results on Abbott SARS-CoV-2 IgG

	All participants		Test result					
			Grayzone		Positive		Negative	
Parameter	*n*	%	*n*	%	*n*	%	*n*	%
Total	367		93		150		124	
Close contact with a case of COVID-19								
Yes	211	57.5	56	60.2	90	60.0	65	52.4
No	154	42.0	37	39.8	58	38.7	59	47.6
Unknown	2	0.5	0	0.0	2	1.3	0	0.0
Main type of patient contact[a]								
Daily contact with known/suspected COVID-19 patients	88	24.0	22	23.7	40	26.7	26	21.0
Daily contact with patients without COVID-19	218	59.4	56	60.2	88	58.7	74	59.7
No patient contact	61	16.6	15	16.1	22	14.7	24	19.4
Symptoms at time of previous PCR test								
No symptoms	7	1.9	1	1.1	3	2.0	3	2.4
Had symptoms	358	97.5	92	98.9	145	96.7	121	97.6
Unknown	2	0.5	0	0.0	2	1.3	0	0.0
Severity of symptoms								
Minor symptoms	151	41.1	38	43.2	51	35.2	62	51.2
Significant symptoms	178	48.5	48	54.5	75	51.7	55	45.5
Severe symptoms (hospitalized)	29	7.9	6	6.8	19	13.1	4	3.3
No. of mo since positive PCR test								
Less than 1	8	2.2	1	1.1	6	4.0	1	0.8
1	8	2.2	0	0.0	8	5.3	0	0.0
2	4	1.1	0	0.0	4	2.7	0	0.0
3	1	0.3	0	0.0	1	0.7	0	0.0
4	12	3.3	2	2.2	10	6.7	0	0.0
5	85	23.2	28	30.1	34	22.7	23	18.5
6	222	60.5	54	58.1	77	51.3	91	73.4
7	25	6.8	8	8.6	8	5.3	9	7.3
Unknown	2	0.5	0	0.0	2	1.3	0	0.0
No. of mo since onset of symptoms[b]								
Less than 1	5	1.5	1	1.1	3	2.2	1	0.9
1	8	2.4	0	0.0	8	5.8	0	0.0
2	3	0.9	0	0.0	3	2.2	0	0.0
3	1	0.3	0	0.0	1	0.7	0	0.0
4	12	3.5	2	2.3	10	7.2	0	0.0
5	78	22.9	25	28.4	32	23.2	21	18.4
6	210	61.8	52	59.1	73	52.9	85	74.6
7	23	6.8	8	9.1	8	5.8	7	6.1

[a]Participants were asked which one describes most of their current work.
[b]Excludes 27 participants who were not symptomatic at the time of their positive PCR test.

Appendix 4 Factors associated with Abbott SARS-CoV-2 IgG seronegativity, among participants who had a previously PCR-confirmed infection, including those who had a grayzone result

		Negative result		Unadjusted			Adjusted		
Parameter	All participants (*n*)	*n*	%	OR	95% CI	*P* value[a]	OR	95% CI	*P* value[a]
Total	367	124	33.8						
Age group									
18–29	111	41	36.9	*[b]			*		
30–39	108	43	39.8	1.13	0.66–1.95	0.662	0.96	0.51–1.8	0.893
40–49	79	17	21.5	0.47	0.24–0.89	0.024	0.44	0.21–0.92	0.031
50–59	50	19	38.0	1.05	0.52–2.08	0.897	1.05	0.47–2.36	0.897
60+	19	4	21.1	0.46	0.12–1.35	0.187	0.32	0.08–1.11	0.090
Sex									
Female	269	102	37.9	*			*		
Male	**98**	**22**	**22.4**	**0.47**	**0.28–0.81**	**0.006**	**0.38**	**0.21–0.68**	**0.001**
Ethnicity									
Irish (white)	237	94	39.7	*			*		
Any other white background	44	12	27.3	0.57	0.27–1.14	0.123	0.64	0.29–1.39	0.273
Any Asian background	72	14	19.4	0.37	0.19–0.68	0.002	0.41	0.19–0.81	0.012
Any African/black background	10	2	20.0	0.38	0.05–1.56	0.228	0.48	0.07–2.29	0.392
Other	4	2	50.0	1.52	0.18–12.9	0.677	1.53	0.15–15.7	0.705
Close contact with a case of COVID-19[c]									
Yes	211	65	30.8	0.72	0.46–1.11	0.147	0.67	0.4–1.12	0.127
No	154	59	38.3	*			*		
Main type of patient contact[d]									
No patient contact	61	24	39.3	*			*		
Daily contact non-COVID-19 patients	218	74	33.9	0.79	0.44–1.43	0.435	0.78	0.4–1.56	0.484
Daily contact known or suspected COVID-19 patients	88	26	29.5	0.65	0.21–0.32	0.214	0.84	0.38–1.89	0.679
Severity of symptoms[c]									
No symptoms	7	3	42.9	1.08	0.21–5.05	0.925	1.07	0.17–6.16	0.942
Minor symptoms	151	62	41.1	*			*		
Significant symptoms	178	55	30.9	0.64	0.41–1.01	0.056	0.58	0.34–0.96	0.037
Severe symptoms (hospitalized)	29	4	13.8	0.23	0.06–0.63	0.009	0.26	0.07–0.76	0.023
HCW role									
Administration	24	9	37.5	*			Not included in the model		
Allied health care	42	19	45.2	1.38	0.49–3.94	0.541			
General support	12	5	41.7	1.19	0.28–4.92	0.809			
Health care assistant	32	5	15.6	0.31	0.08–1.06	0.068			
Medical/dental	73	29	39.7	1.10	0.43–2.9	0.846			
Nursing/midwifery	181	55	30.4	0.73	0.31–1.83	0.481			
Other	3	2	66.7	3.33	0.28–78.0	0.353			
No. of mo since PCR positive test[c]									
Less than 1	8	1	12.5	NA	NA	NA	0.23	0.01–1.6	0.204
1	8	0	0.0	NA	NA	NA	NA	NA	NA
2	4	0	0.0	NA	NA	NA	NA	NA	NA
3	1	0	0.0	NA	NA	NA	NA	NA	NA
4	12	0	0.0	NA	NA	NA	NA	NA	NA
5	85	23	27.1	*			*		
6	222	91	41.0	1.87	1.09–3.29	0.025	1.95	1.07–3.64	0.032
7	25	9	36.0	1.52	0.57–3.87	0.389	2.07	0.72–5.9	0.172

[a]*P* values were calculated using the chi-square test, results for significant associations are highlighted in bold.
[b]*, reference category.
[c]Excludes two unknowns.
[d]Participants were asked which type of patient contact describes most of their current work (excludes 5 unknowns).

ACKNOWLEDGMENTS

We acknowledge the study steering group who planned the study and critically evaluated the manuscript, the study team who coordinated the running of the study in each hospital, the hospital management at both sites for their support for the study, and the staff of both hospitals who participated. We would especially like to acknowledge the phlebotomy departments in each hospital for facilitating the sampling of almost 6,000 participants; the microbiology, virology, and biochemistry laboratories in each hospital for validating the assays and processing the samples on two different assays; the National Virus Reference Laboratory of Ireland for additional testing; and the human resources department for their help with denominator data.

This work was supported by the Irish Health Service Executive COVID-19 budget. Work by N.C. is funded partly by a Science Foundation Ireland (SFI) grant, grant code 20/SPP/3685.

We declare no conflicts of interest.

REFERENCES

1. Watson J, Richter A, Deeks J. 2020. Testing for SARS-CoV-2 antibodies. BMJ 370:m3325. https://doi.org/10.1136/bmj.m3325.
2. Walsh EE, Frenck RW, Falsey AR, Kitchin N, Absalon J, Gurtman A, Lockhart S, Neuzil K, Mulligan MJ, Bailey R, Swanson KA, Li P, Koury K, Kalina W, Cooper D, Fontes-Garfias C, Shi P-Y, Türeci Ö, Tompkins KR, Lyke KE, Raabe V, Dormitzer PR, Jansen KU, Şahin U, Gruber WC. 2020. Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. N Engl J Med 383:2439–2450. https://doi.org/10.1056/NEJMoa2027906.
3. Vabret N, Britton GJ, Gruber C, Hegde S, Kim J, Kuksin M, Levantovsky R, Malle L, Moreira A, Park MD, Pia L, Risson E, Saffern M, Salomé B, Esai Selvan M, Spindler MP, Tan J, van der Heide V, Gregory JK, Alexandropoulos K, Bhardwaj N, Brown BD, Greenbaum B, Gümüş ZH, Homann D, Horowitz A, Kamphorst AO, Curotto de Lafaille MA, Mehandru S, Merad M, Samstein RM, Sinai Immunology Review Project. 2020. Immunology of COVID-19: current state of the science. Immunity 52:910–941. https://doi.org/10.1016/j.immuni.2020.05.002.
4. Hanrath AT, Payne BAI, Duncan CJA. 2020. Prior SARS-CoV-2 infection is associated with protection against symptomatic reinfection. J Infect 82:e29–e30. https://doi.org/10.1016/j.jinf.2020.12.023.
5. Post N, Eddy D, Huntley C, van Schalkwyk MCI, Shrotri M, Leeman D, Rigby S, Williams SV, Bermingham WH, Kellam P, Maher J, Shields AM, Amirthalingam G, Peacock SJ, Ismail SA. 2020. Antibody response to SARS-CoV-2 infection in humans: a systematic review. PLoS One 15:e0244126. https://doi.org/10.1371/journal.pone.0244126.
6. Ibarrondo FJ, Fulcher JA, Goodman-Meza D, Elliott J, Hofmann C, Hausner MA, Ferbas KG, Tobin NH, Aldrovandi GM, Yang OO. 2020. Rapid decay of anti–SARS-CoV-2 antibodies in persons with mild Covid-19. N Engl J Med 383:1085–1087. https://doi.org/10.1056/NEJMc2025179.
7. Patel MM, Thornburg NJ, Stubblefield WB, Talbot HK, Coughlin MM, Feldstein LR, Self WH. 2020. Change in antibodies to SARS-CoV-2 over 60 days among health care personnel in Nashville, Tennessee. JAMA 324:1781–1782. https://doi.org/10.1001/jama.2020.18796.
8. Gudbjartsson DF, Norddahl GL, Melsted P, Gunnarsdottir K, Holm H, Eythorsson E, Arnthorsson AO, Helgason D, Bjarnadottir K, Ingvarsson RF, Thorsteinsdottir B, Kristjansdottir S, Birgisdottir K, Kristinsdottir AM, Sigurdsson MI, Arnadottir GA, Ivarsdottir EV, Andresdottir M, Jonsson F, Agustsdottir AB, Berglund J, Eiriksdottir B, Fridriksdottir R, Gardarsdottir EE, Gottfredsson M, Gretarsdottir OS, Gudmundsdottir S, Gudmundsson KR, Gunnarsdottir TR, Gylfason A, Helgason A, Jensson BO, Jonasdottir A, Jonsson H, Kristjansson T, Kristinsson KG, Magnusdottir DN, Magnusson OT, Olafsdottir LB, Rognvaldsson S, Le Roux L, Sigmundsdottir G, Sigurdsson A, Sveinbjornsson G, Sveinsdottir KE, Sveinsdottir M, Thorarensen EA, Thorbjornsson B, Thordardottir M, Saemundsdottir J, et al. 2020. Humoral immune response to SARS-CoV-2 in Iceland. N Engl J Med 383:1724–1734. https://doi.org/10.1056/NEJMoa2026116.
9. Wajnberg A, Amanat F, Firpo A, Altman DR, Bailey MJ, Mansour M, McMahon M, Meade P, Mendu DR, Muellers K, Stadlbauer D, Stone K, Strohmeier S, Simon V, Aberg J, Reich DL, Krammer F, Cordon-Cardo C. 2020. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science 370:1227–1230. https://doi.org/10.1126/science.abd7728.
10. National SARS-CoV-2 Serology Assay Evaluation Group. 2020. Performance characteristics of five immunoassays for SARS-CoV-2: a head-to-head benchmark comparison. Lancet Infect Dis 20:1390–1400. https://doi.org/10.1016/S1473-3099(20)30634-4.
11. Muecksch F, Wise H, Batchelor B, Squires M, Semple E, Richardson C, et al. 2020. Longitudinal analysis of clinical serology assay performance and neutralising antibody levels in COVID19 convalescents [Internet]. medRxiv. https://doi.org/10.1101/2020.08.05.20169128.
12. Lumley SF, Wei J, O'Donnell D, Stoesser NE, Matthews PC, Howarth A, et al. 2021. The duration, dynamics and determinants of SARS-CoV-2 antibody responses in individual healthcare workers. Clin Infect Dis 73:e699–e709. https://doi.org/10.1093/cid/ciab004.
13. Kerr C, Allen N, Hughes G, Kelly M, O'Rourke F, Lynagh Y, Dunne J, Crowley B, Conlon N, Bergin C. 2021. SARS-CoV-2 anti-nucleocapsid assay performance in healthcare workers at baseline and 6 months. Ir J Med Sci. https://doi.org/10.1007/s11845-021-02700-5.
14. Allen N, Ní Riain U, Conlon N, Ferenczi A, C Martin AI, Domegan L, Walsh C, Doherty L, O'Farrelly C, Higgins E, Kerr C, McGrath J, PRECISE Study Steering Group, Fleming C, Bergin C. 2021. Prevalence of antibodies to SARS-CoV-2 in Irish hospital healthcare workers. Epidemiol Infect 149:e157. https://doi.org/10.1017/S0950268821000984.
15. Food and Drug Administration. 2021. SARS-CoV-2 IgG Architect—instructions for use, FDA. Food and Drug Administration, Silver Spring, MD. https://www.fda.gov/media/137383/download.
16. ARCHITECT System. 2021. SARS-COV-2IgG/IgM e- Assay CD-ROM - WW (excluding US) list number 6S14-03. Abbott Diagnostics, IL, USA.
17. Elecsys. Anti-SARS-CoV-2 immunoassay for the qualitative detection of antibodies (incl. IgG) against SARS-CoV-2, Roche Diagnostics, Basel, Switzerland https://diagnostics.roche.com/global/en/products/params/elecsys-anti-sars-cov-2.html.
18. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, Wang X, Yuan J, Li T, Li J, Qian S, Hong C, Wang F, Liu Y, Wang Z, He Q, Li Z, He B, Zhang T, Fu Y, Ge S, Liu L, Zhang J, Xia N, Zhang Z. 2020. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis 71:2027–2034. https://doi.org/10.1093/cid/ciaa344.
19. Algaissi A, Alfaleh MA, Hala S, Abujamel TS, Alamri SS, Almahboub SA, Alluhaybi KA, Hobani HI, Alsulaiman RM, AlHarbi RH, ElAssouli M-ZA, Alhabbab RY, AlSaieedi AA, Abdulaal WH, Al-Somali AA, Alofi FS, Khogeer AA, Alkayyal AA, Mahmoud AB, Almontashiri NAM, Pain A, Hashem AM. 2020. SARS-CoV-2 S1 and N-based serological assays reveal rapid seroconversion and induction of specific antibody response in COVID-19 patients. Sci Rep 10:16561. https://doi.org/10.1038/s41598-020-73491-5.
20. Pollán M, Pérez-Gómez B, Pastor-Barriuso R, Oteo J, Hernán MA, Pérez-Olmeda M, Sanmartín JL, Fernández-García A, Cruz I, Fernández de Larrea N, Molina M, Rodríguez-Cabrera F, Martín M, Merino-Amador P, León Paniagua J, Muñoz-Montalvo JF, Blanco F, Yotti R, Blanco F, Gutiérrez Fernández R, Martín M, Mezcua Navarro S, Molina M, Muñoz-Montalvo JF, Salinero Hernández M, Sanmartín JL, Cuenca-Estrella M, Yotti R, León Paniagua J, Fernández de Larrea N, Fernández-Navarro P, Pastor-Barriuso R, Pérez-Gómez B, Pollán M, Avellón A, Fedele G, Fernández-García A, Oteo Iglesias J, Pérez Olmeda MT, Cruz I, Fernandez Martinez ME, Rodríguez-Cabrera FD, Hernán MA, Padrones Fernández S, Rumbao

Aguirre JM, Navarro Marí JM, Palop Borrás B, Pérez Jiménez AB, Rodríguez-Iglesias M, Calvo Gascón AM, et al. 2020. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. Lancet 396:535–544. https://doi.org/10.1016/S0140-6736(20)32266-2.
21. Petersen LR, Sami S, Vuong N, Pathela P, Weiss D, Morgenthau BM, Henseler RA, Daskalakis DC, Atas J, Patel A, Lukacs S, Mackey L, Grohskopf LA, Thornburg N, Akinbami LJ. 2020. Lack of antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a large cohort of previously infected persons. Clin Infect Dis 2020:ciaa1685. https://doi.org/10.1093/cid/ciaa1685.
22. Kaufman HW, Chen Z, Meyer WA, Wohlgemuth JG. 2021. Insights from patterns of SARS-CoV-2 immunoglobulin G serology test results from a National Clinical Laboratory, United States, March–July 2020. Popul Health Manag 24:535–542. https://doi.org/10.1089/pop.2020.0256.
23. Pelleau S, Woudenberg T, Rosado J, Donnadieu F, Garcia L, Obadia T, Gardais S, Elgharbawy Y, Velay A, Gonzalez M, Yves Nizou J, Khalil N, Zannis K, Cockram S, Hélène Merkling S, Meola S, Kerneis S, Terrier B, de Seze J, Planas D, Schwartz O, Dejardin F, Petres S, von Platen S, Arowas L, Perrin de Facci L, Duffy D, Ní Cheallaigh C, Conlon N, Townsend L, Auerswald H, Backovic M, Hoen B, Fontanet A, Mueller I, Fafi-Kremer S, Bruel T, White M. 2021. Serological reconstruction of COVID-19 epidemics through analysis of antibody kinetics to SARS-CoV-2 proteins. medRxiv. https://doi.org/10.1101/2021.03.04.21252532.
24. Weidner L, Gänsdorfer S, Unterweger S, Weseslindtner L, Drexler C, Farcet M, Witt V, Schistal E, Schlenke P, Kreil TR, Jungbauer C. 2020. Quantification of SARS-CoV-2 antibodies with eight commercially available immunoassays. J Clin Virol 129:104540. https://doi.org/10.1016/j.jcv.2020.104540.
25. Food and Drug Administration. 2021. EUA authorized serology test performance. Food and Drug Administration, Silver Spring, MD. https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-test-performance.
26. Harley K, Gunsolus IL. 2020. Comparison of the clinical performances of the Abbott Alinity IgG, Abbott Architect IgM, and Roche Elecsys total SARS-CoV-2 antibody assays. J Clin Microbiol 59:e02104-20. https://doi.org/10.1128/JCM.02104-20.
27. Kumar N, Bhartiya S, Singh T. 2021. Duration of anti-SARS-CoV-2 antibodies much shorter in India. Vaccine 39:886–888. https://doi.org/10.1016/j.vaccine.2020.10.094.
28. Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, Adriano A, Beese S, Dretzke J, Ferrante di Ruffano L, Harris IM, Price MJ, Dittrich S, Emperador D, Hooft L, Leeflang MM, Van den Bruel A. 2020. Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Database Syst Rev 6:CD013652. https://doi.org/10.1002/14651858.CD013652.
29. Health Information and Quality Authority. 2021. Advice to the National Public Health Emergency Team. Duration of immunity following SARS-CoV-2 infection. Health Information and Quality Authority, Cork, Ireland. https://www.hiqa.ie/sites/default/files/2021-03/Duration-of-protective-immunity_Advice-to-NPHET-24%20Feb-2021.pdf.
30. Allen N. Prevalence of antibodies to SARS-CoV-2 in Irish healthcare workers phase 1 October 2020. 2021, HSE Health Protection Surveillance Centre, Ireland. https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/research/precise/PRECISE%20Study%20Phase%201%20Interim%20Report%20January%202021.pdf.
31. Dean AG, Sullivan KM, Soe MM. OpenEpi: open source epidemiologic statistics for public health, version 3.01, www.OpenEpi.com, updated 6 April 2013, accessed 2021.

Appendix 2.4 Allen N et al. *Frontiers in Medicine* (2022)

frontiers in Medicine

ORIGINAL RESEARCH
published: 04 February 2022
doi: 10.3389/fmed.2021.758118

Prevalence of Antibodies to SARS-CoV-2 Following Natural Infection and Vaccination in Irish Hospital Healthcare Workers: Changing Epidemiology as the Pandemic Progresses

Niamh Allen[1*], *Melissa Brady*[2,3], *Una Ní Riain*[4], *Niall Conlon*[5,6], *Lisa Domegan*[3], *Antonio Isidro Carrion Martin*[7], *Cathal Walsh*[2,8,9], *Lorraine Doherty*[2], *Eibhlin Higgins*[6], *Colm Kerr*[1], *on behalf of the PRECISE Study Steering Group*, *Colm Bergin*[1,10†] *and Catherine Fleming*[10†]

[1] *Department of GU Medicine and Infectious Diseases (GUIDE), St. James's Hospital, Dublin, Ireland,* [2] *European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden,* [3] *Health Protection Surveillance Centre (HPSC), Dublin, Ireland,* [4] *Department of Microbiology, University Hospital Galway, Galway, Ireland,* [5] *Department of Immunology, St. James's Hospital, Dublin, Ireland,* [6] *Department of Clinical Medicine, Trinity College, Dublin, Ireland,* [7] *Division of Preventive Medicine and Public Health, Department of Public Health Sciences, University of Murcia School of Medicine, Murcia, Spain,* [8] *Health Research Institute and MACSI, University of Limerick, Limerick, Ireland,* [9] *MSA and NCRE, St James's Hospital, Dublin, Ireland,* [10] *Department of Infectious Diseases, University Hospital Galway, Galway, Ireland*

Background: In October 2020 SARS-CoV-2 seroprevalence among hospital healthcare workers (HCW) of two Irish hospitals was 15 and 4. 1%, respectively. We compare seroprevalence in the same HCW population 6 months later, assess changes in risk factors for seropositivity with progression of the pandemic and serological response to vaccination.

Methods: All staff of both hospitals (*N* = 9,038) were invited to participate in an online questionnaire and SARS-CoV-2 antibody testing in April 2021. We measured anti-nucleocapsid and anti-spike antibodies. Frequencies and percentages for positive SARS-CoV-2 antibodies were calculated and adjusted relative risks for participant characteristics were calculated using multivariable regression analysis.

Results: Five thousand and eighty-five HCW participated. Seroprevalence increased to 21 and 13%, respectively; 26% of infections were previously undiagnosed. Black ethnicity (aRR 1.7, 95% CI 1.3–2.2, $p < 0.001$), lower level of education (aRR 1.4 for secondary level education, 95% CI 1.1–1.8, $p = 0.002$), living with other HCW (aRR 1.2, 95% CI 1.0–1.4, $p = 0.007$) were significantly associated with seropositivity. Having direct patient contact also carried a significant risk being a healthcare assistant (aRR 1.8, 95% CI 1.3–2.3, $p < 0.001$), being a nurse (aRR 1.4, 95% CI 1.0–1.8, $p = 0.022$), daily contact with COVID-19 patients (aRR 1.4, 95% CI 1.1–1.7, $p = 0.002$), daily contact with patients without suspected or confirmed COVID-19 (aRR 1.3, 95% CI 1.1–1.5, $p = 0.013$). Breakthrough infection occurred in 23/4,111(0.6%) of fully vaccinated participants; all had anti-S antibodies.

OPEN ACCESS

Edited by:
Diamantis Plachouras, European Centre for Disease Prevention and Control (ECDC), Sweden

Reviewed by:
Qilin Yang, The Second Affiliated Hospital of Guangzhou Medical University, China
Oyewale Tomori, Redeemer's University, Nigeria

***Correspondence:**
Niamh Allen
allen.niamh@gmail.com

† *These authors have contributed equally to this work*

Specialty section:
This article was submitted to Infectious Diseases – Surveillance, Prevention and Treatment, a section of the journal Frontiers in Medicine

Received: *13 August 2021*
Accepted: *31 December 2021*
Published: *04 February 2022*

Citation:
Allen N, Brady M, Ní Riain U, Conlon N, Domegan L, Carrion Martin AI, Walsh C, Doherty L, Higgins E, Kerr C, Bergin C and Fleming C (2022) Prevalence of Antibodies to SARS-CoV-2 Following Natural Infection and Vaccination in Irish Hospital Healthcare Workers: Changing Epidemiology as the Pandemic Progresses. Front. Med. 8:758118. doi: 10.3389/fmed.2021.758118

Conclusion: The increase in seroprevalence reflects the magnitude of the third wave of the pandemic in Ireland. Genomic sequencing is needed to apportion risk to the workplace vs. the household/community. Concerted efforts are needed to mitigate risk factors due to ethnicity and lower level of education, even at this stage of the pandemic. The undiagnosed and breakthrough infections call for ongoing infection prevention and control measures and testing of HCW in the setting of close contact. Vaccinated HCW with confirmed infection should be actively assessed, including SARS-CoV-2 whole genome sequencing (WGS), serology testing and assessment of host determinants, to advance understanding of the reasons for breakthrough infection.

Keywords: COVID-19 seroprevalence, SARS-CoV-2 seroprevalence, healthcare worker (HCW), SARS-CoV-2 antibodies, hospital seroprevalence SARS-CoV-2

INTRODUCTION

SARS-CoV-2 Infection in Hospital Healthcare Workers

Healthcare workers, and those they live with, are at increased risk of contracting SARS-CoV-2 viral infection (1–3). Detectable antibody to SARS-CoV-2 is an excellent indicator of previous SARS-CoV-2 infection (4). A high proportion of the SARS-CoV-2 infections notified worldwide have been in hospital healthcare workers (HCW) and antibody seroprevalence has been shown to be higher in HCW than in the general population (5–7). Understanding the transmission and potential immunity dynamics of SARS-CoV-2 in hospitals is important in mitigating transmission at hospital level and adds valuable information to the growing evidence on the transmission patterns of COVID-19 among HCW.

Antibody Response Following Infection and Vaccination

Natural infection has been shown to produce humoral and cellular immunity and whilst this may decline over time, durable memory responses are seen (8, 9). Vaccines have been shown to be protective both against infection and against symptomatic disease (10–13). Robust B and T cell responses to vaccination have been shown for both mRNA vaccines and viral vector vaccines (14). Antibody response has been shown to correlate with protective immunity against infection (15).

The spike (S) and nucleocapsid (N) proteins are two of the main immunogens of the coronavirus proteins (16). Commercial SARS-CoV-2 antibody assays can detect antibodies to these structural proteins. Natural infection with SARS-CoV-2 elicits antibodies against the spike protein and the nucleocapsid protein (17). Currently available vaccines against the SARS-CoV-2 virus target the spike protein only (18, 19). The detection of anti-N antibodies allows vaccine-induced seroconversion to be distinguished from antibodies elicited by natural infection (20).

Study Sites

Hospital 1 is a tertiary referral hospital in the inner city of Dublin, the capital city of Ireland (population 1.2 million) and has almost 4,700 employees and just over 1,000 beds. It is one of the largest acute hospitals in Dublin city. Hospital 2 is a comparable tertiary referral hospital with almost 4,400 employees and over 500 beds, located in Galway, in the West of Ireland (population 80,000). It is the main acute hospital serving the city of Galway. Both hospitals received patients with COVID-19 infection throughout the first wave of the pandemic in Ireland, and breakdown by ward and specialty is similar. The community incidence of COVID-19 in County Galway was significantly lower than in County Dublin during the first and second waves of the pandemic in Ireland (21). During the third wave of the pandemic in Ireland (peak January 2021) the 14-day incidence for Galway approached that of Dublin (22). The first part of this study was conducted in October 2020, during the second wave of the pandemic in Ireland, and prior to the roll-out of COVID-19 vaccination. It showed an overall SARS-CoV-2 seroprevalence of 15% in Hospital 1 and 4.1% in Hospital 2, respectively. Almost 40% of infections had been previously undiagnosed (6, 23). The HCW seroprevalence was six times higher than community seroprevalence (5). By the start of April 2021(decline of third wave) the cumulative incidence of PCR-confirmed infections in HCW in Hospital 1 and 2 had risen to 18.5 and 9.2%, respectively.

The purpose of this repeat cross-sectional study was to re-assess the prevalence of anti-SARS-CoV-2 antibodies in HCW in these two hospitals following the third, and largest, wave of the pandemic in Ireland, and assess changes in demographic and work-related risk factors. We also aimed to assess serological response to COVID-19 vaccination in the vaccinated sub-group, and to quantify breakthrough infections.

MATERIALS AND METHODS

Study Design and Participants

This was a cross-sectional study of the seroprevalence of circulating antibodies to SARS-CoV-2, carried out from the 19th to 28th April 2021. All staff members of both hospitals (N = 9,038) were invited to participate in an online self-administered consent process and online questionnaire, followed by blood sampling for SARS-CoV-2 antibody testing in April 2021, in the same manner as October 2020 (6). Electronic consent and patient reported outcomes were captured using an eClinical platform Castor (24). Information collected in the questionnaire

included demographic information, contact details, place and type of work, level of contact with patients, previous COVID-19 symptoms and testing, history of close contact with a confirmed case of COVID-19, living arrangements and history of COVID-19 vaccination, including dates and type of vaccine. Blood samples were processed anonymously. Results were sent by text message to all participants on an opt-out basis. Results were discussed in person with any participant who requested this.

All vaccinated study participants received their COVID-19 vaccine as part of a two-dose regimen of the Comirnaty (Pfizer/BioNTech) vaccine, the Vaxzevria (formerly AstraZeneca) vaccine or the Moderna vaccine. A participant was considered partially vaccinated at ≥14 days after receipt of the first dose of vaccination, and fully vaccinated ≥14 days after receipt of the second dose of vaccination in line with Irish and international guidelines (25, 26).

Laboratory Methods

All samples were tested using the Roche Elecsys anti-SARS-CoV-2 and the Roche Elecsys anti-SARS-CoV-2 S immunoassays detecting total antibodies (including IgG) to the nucleocapsid and spike proteins of the SARS-CoV-2 virus, respectively (27). Thresholds for positive results were as per manufacturers' guidelines (27, 28). Participants with detectable anti-N antibodies were presumed to have had previous natural infection. Participants with detectable anti-S antibodies, and no reported history of COVID-19 vaccination were also presumed to have had natural infection. Participants with detectable anti-S antibodies and a history of COVID-19 vaccination were presumed to have these anti-S antibodies in response to vaccination.

Statistical Analysis

Frequencies and percentages were calculated for sociodemographic, epidemiological, and clinical characteristics. Participants were deemed seropositive (i.e., assumed to have had past infection with SARS-CoV-2) if they had detectable anti-N antibodies, or if they had detectable anti-S antibodies but had not been previously vaccinated. Characteristics of those who were seropositive were compared to those who were not seropositive, using the chi-square test. Univariable logistic regression was used to calculate relative risks along with their 95% confidence intervals to assess the association between SARS-CoV-2 seropositivity and characteristics of the study participants. Multivariable logistic regression analysis was conducted to control for negative and positive confounding and to calculate adjusted relative risks (aRR). No explicit finite population correction or reweighting was carried out. All analysis was conducted in Stata 15.1 (StataCorp LCC. 2019. College Station, TX 77845: USA).

Ethical Approval

Ethical approval was obtained from the National Research Ethics Committee (NREC) for COVID-19, Study Number 20-NREC. COV-101 (29).

Funding

This work was supported financially by the Irish Health Service Executive COVID-19 budget.

RESULTS

SARS-CoV-2 Seroprevalence (Past Infection)

Participation Rates and Demographics

In total 5,085 HCW participated (56% of invited staff). These 5,085 participants included 3,313 HCW who also participated in the first phase. On combined hospital data, 78% of participants were female. Median age was 40 years (IQR 30–49). By ethnicity 75% of participants were white Irish, 12% were Asian, and 2.3% were of African or any other black background. Ninety-one percent of participants lived with other people and 31% lived with other HCW. The highest proportion (37%) of participants were nursing staff, 21% were allied healthcare staff, 14% were doctors, 13% were administration staff, 7.2% were general support staff and 5.7% health care assistants (HCA). By hospital, age and sex of participants were similar while ethnicity differed slightly (**Table 1a**). Participation by staff grouping was similar in both hospitals and participants' working roles broadly reflected the overall breakdown by role of the staff in both hospitals [For details on participation by HCW role see **Supplementary Tables A–D** in Annex, main PRECISE 2 Study Report (30)].

Previous Exposure, Symptoms and Testing

Hospital 1 staff had a higher percentage of previously confirmed SARS-CoV-2 infection; 18% of participants in Hospital 1 and 14% of participants in Hospital 2 reported that they had previously tested positive for COVID-19 infection by PCR. **Table 1b** shows the COVID-19 related characteristics of the participants by hospital.

Seroprevalence of Antibodies to SARS-CoV-2

The overall seroprevalence of antibodies to SARS-CoV-2, indicative of past natural infection, was 21% in Hospital 1 and 13% in Hospital 2. Seroprevalence was higher in those with direct patient contact (especially those working with patients with COVID-19 infection). Seroprevalence by degree of patient contact, sociodemographic characteristics and COVID-19 characteristics are shown in **Tables 2a,b**. (Breakdown by hospital is shown in **Supplementary Tables 2c–f** in Annex). By professional subgroup, the highest seroprevalence was seen amongst HCAs (32%), followed by general support staff (22%) and nurses/midwives (21%) (A detailed breakdown of general support staff by hospital is shown in **Supplementary Table 2g** in Annex).

SARS-CoV-2 Seropositivity (Due to Natural Infection) by Previous Diagnosis and Symptoms

Sixteen percent (812/5,085) of participants reported having had a PCR-confirmed infection with COVID-19 at some stage. Of these, 82% (663/812) were seropositive. Twenty-one percent (172/812) were asymptomatic at the time of their positive PCR,

TABLE 1a | Participant characteristics by hospital and total number of participants, April 2021.

Participant characteristics		Hospital 1 (N = 2,945)		Hospital 2 (N = 2,140)		*P*-value*	Total (N = 5,085)	
		n	%	*n*	%		*n*	%
Age groups	18–29	653	22	455	21	0.431	1,108	22
	30–39	765	26	565	26		1,330	26
	40–49	811	28	603	28		1,414	28
	50–59	565	19	386	18		951	19
	≥60	151	5.1	131	6.1		282	5.5
Sex	Female	2,278	77	1,681	79	0.309	3,959	78
	Male	667	23	459	21		1,126	22
Ethnicity	Irish (White)	2,091	71	1,707	80	**<0.001**	3,798	75
	Any other white background	257	8.7	219	10		476	9.4
	Asian background	470	16	129	6.0		599	12
	African/other black background	69	2.3	48	2.2		117	2.3
	Other	58	2.0	37	1.7		95	1.9
Country of birth	Ireland	2,025	69	1, 605	75	**<0.001**	3,630	71
	United Kingdom	134	4.6	161	7.5		295	5.8
	India	225	7.6	68	3.2		293	5.8
	Philippines	198	6.7	16	0.7		214	4.2
	Poland	26	0.9	59	2.8		85	1.7
	USA	21	0.7	34	1.6		55	1.1
	Other	316	11	197	9.2		513	10
Education	Primary	20	0.7	2	0.1	**<0.001**	22	0.4
	Secondary	409	14	200	9.3		609	12
	Third level	1,280	43	964	45		2,244	44
	Post-graduate	1,236	42	974	46		2,210	43
Role	Admin	403	14	273	13	**<0.001**	676	13
	Medical/dental	357	12	356	17		713	14
	Nursing/ midwifery	1,097	37	794	37		1,891	37
	Allied health	612	21	432	20		1,044	21
	General support	243	8.3	122	5.7		365	7.2
	Health care assistant	179	6.1	112	5.2		291	5.7
	Other	54	1.8	51	2.4		105	2.1
Lives with	Alone	270	9.2	194	9.1	0.603	464	9.1
	With others	2,667	90.6	1,943	90.8		4,610	90.7
	Missing	8	0.3	3	0.1		11	0.2
Lives with HCW	Yes	928	32	643	30	0.284	1,571	31
	No	1,964	67	1,448	68		3,412	67
	Missing	53	1.8	49	2.3		102	2.0

*Calculated using the chi-square test. Statistically significant findings in bold.

Table 2b. Seroprevalence among those that were symptomatic (576/640; 90%) was significantly higher than seroprevalence among those who were asymptomatic at the time of their confirmed COVID-19 infection (87/172; 51%; $p < 0.001$). Nineteen percent (169/898) of those with detectable antibodies had never experienced symptoms consistent with COVID-19 infection.

Undiagnosed SARS-CoV-2 Infection

In total, 898 participants were seropositive (due to natural infection). Of these, 235/898 (26%) had never been diagnosed with COVID-19 infection, representing 4.6% (235/5,085) of the total study population. Just over half of those with undiagnosed infection (121/235; 51%) had experienced COVID-19 like symptoms at some stage; of those 74% (89/121) had experienced mild symptoms and 26% (32/121) had experienced moderate symptoms. This proportion of symptomatic undiagnosed participants who experienced only mild symptoms (89/121, 74%) was much higher than the proportion of symptomatic diagnosed participants who experienced only mild symptoms (226/576; 39%) (**Figure 1**) [**Supplementary Table A** in Annex, main report (30)]. Most participants with undiagnosed infection reported

TABLE 1b | COVID-19 related characteristics by hospital and total number of participants, April 2021.

Participant characteristics		Hospital 1 (N = 2,945)		Hospital 2 (N = 2,140)		*P*-value*	Total (N = 5,085)	
		n	%	*n*	%		*n*	%
Daily contact with COVID-19 patients	Contact with COVID-19 patients	726	25	410	19	**<0.001**	1,136	22
	Contact with patients without COVID-19	1,362	46	1,138	53		2,500	49
	No patient contact	857	29	592	28		1,449	28
Previous COVID-19 symptoms (ever)	No symptoms	1,571	53	1,342	63	**<0.001**	2,913	57
	Had symptoms	1,374	47	797	37		2,171	43
	Missing	0	0.0	1	0.0		1	0.0
Severity of symptoms	No symptoms	1,571	53	1,342	63	**<0.001**	2,913	57
	Mild symptoms	932	32	570	27		1,502	30
	Significant symptoms	420	14	201	9.4		621	12
	Severe (hospitalized)	21	0.7	26	1.2		47	0.9
	Type of symptoms unknown	1	0.0	0	0.0		1	0.0
	Missing	0	0.0	1	0.0		1	0.0
Previous positive COVID-19 PCR test	No	2,427	82	1,846	86	**<0.001**	4,273	84
	Yes	518	18	294	14		812	16
Symptoms at time of previous positive PCR test	No	95	18	77	26	**<0.001**	172	21
	Yes	423	82	217	74		640	79
Severity of symptoms at time of PCR test	No symptoms	95	18	77	26	**<0.001**	172	21
	Mild symptoms	162	31	94	32		256	32
	Significant symptoms	248	48	103	35		351	43
	Severe (hospitalized)	12	2.3	20	6.8		32	3.9
	Missing	1	0.2	0	0.0		1	0.1

*Calculated using the chi-square test. *Statistically significant findings in bold.*

daily patient contact in their role (192/235; 82%). Detailed analysis of undiagnosed infection by HCW role and by hospital is shown in **Supplementary Table 2i** in Annex.

Risk Factors for SARS-CoV-2 Antibody Positivity

Characteristics of those participants who were seropositive compared with those who were seronegative for both hospitals combined are shown in **Tables 2a,b**. On multivariable analysis of the combined hospital data, the adjusted relative risk (aRR) of seropositivity was statistically significant for the following characteristics: working in Hospital 1 (aRR 1.5, 95% CI 1.3–1.8, $p < 0.001$), being a healthcare assistant (aRR 1.8, 95% CI 1.3-2.3, p<0.001), being of African or other black background (aRR 1.7, 95% CI 1.3–2.2, $p < 0.001$), lower level of education (aRR 1.4 for secondary level education, 95% CI 1.1–1.8, $p = 0.002$), being a nurse (aRR 1.4, 95% CI 1.0–1.8, $p = 0.022$), daily contact with COVID-19 patients (aRR 1.4, 95% CI 1.1–1.7, $p = 0.002$), daily contact with patients without suspected or confirmed COVID-19 (aRR 1.3, 95% CI 1.1–1.5, $p = 0.013$), being 18–29 years of age (aRR 1.3, 95% CI 1.1–1.6, $p = 0.002$), being male (aRR 1.2, 95% CI 1.0–1.4, $p = 0.016$), and living with other HCW (aRR 1.2, 95% CI 1.0–1.4, $p = 0.007$), **Table 3a**. Multivariable analysis by hospital is shown in **Supplementary Tables 3b,c** in Annex.

Antibody Response to Vaccination

In total, 80% (4,111/5,085) of participants were fully vaccinated and a further 14% (716/5,085) were partially vaccinated. All fully vaccinated participants had received the Pfizer vaccine and the majority of partially vaccinated participants (681/716, 95%) had received the Vaxzevria vaccine [due to timing of vaccine roll-out (which commenced with the Pfizer vaccine) and due to the longer dosing interval of the Vaxzevria vaccine (26)]. Vaccination uptake by ethnicity was similar, with 142/3,661 (3.9%, 95% CI 3.3–4.6) of White Irish participants being unvaccinated, compared to 30/571 (5.2%, 95% CI 3.7–7.4) of Asian participants and 7/110 (6.4%, 95% CI 3.1–13) of Black participants. All fully vaccinated participants (4,111/4,111, 100%) and 99.6% (713/716) of partially vaccinated participants had detectable anti-S antibodies.

SARS-CoV-2 Infection Post-vaccination

In total, 116 participants reported PCR-confirmed SARS-CoV-2 infection since vaccination; of those 23/116 (20%) were fully vaccinated, representing 0.6% (23/4,111) of all fully vaccinated participants. There were 93 infections in partially vaccinated participants representing 93/716 (13%; 95% CI 11–16) of partially vaccinated participants having had a PCR-confirmed infection post-vaccination compared to 23/4,111 (0.6%; 95% CI 0.4–0.8) of fully vaccinated participants [p-value = <0.001 (chi-square)]. All fully and partially vaccinated participants with infection post-vaccination had anti-spike antibodies detected.

Of the 23 breakthrough infections in fully vaccinated participants, all had received the Pfizer vaccine (It is noted that recipients of other vaccines in our study were not

TABLE 2a | Prevalence of SARS-CoV-2 seropositivity by participant characteristics, both hospitals, April 2021.

Participant characteristics		Total	SARS-CoV-2 seropositive participants		
		N	*n*	% (95% CI)	*p*-value*
All participants		5,085	898	18 (17–19)	
Hospital	Hospital 1	2,945	623	21 (20–23)	**<0.001**
	Hospital 2	2,140	275	13 (11–14)	
Age groups (years)	18–29	1,108	249	22 (20–25)	**<0.001**
	30–39	1,330	238	18 (16–20)	
	40–49	1,414	208	15 (13–17)	
	50–59	951	158	17 (14–19)	
	Over 60	282	45	16 (12–21)	
Sex	Female	3,959	669	17 (16–18)	**0.008**
	Male	1,126	229	20 (18–23)	
Ethnicity	Irish (White)	3,798	595	16 (15–17)	**<0.001**
	Any other white background	476	94	20 (16–24)	
	Asian background	599	148	25 (21–28)	
	African or any other black background	117	39	33 (25–43)	
	Other	95	22	23 (15–33)	
Country of birth	Ireland	3,630	567	16 (14–17)	**<0.001**
	United Kingdom	295	44	15 (11–20)	
	India	293	76	26 (21–31)	
	Philippines	214	54	25 (20–32)	
	Poland	85	20	24 (15–34)	
	USA	55	11	20 (10–33)	
	Romania	45	15	33 (20–49)	
	Nigeria	35	19	54 (31–71)	
	Other	433	92	21 (17–25)	
Education	Primary	22	7	32 (14–55)	**<0.001**
	Secondary	609	133	22 (19–25)	
	Third level	2,244	434	19 (18–21)	
	Post-graduate	2,210	324	15 (13–16)	
Role	Admin	676	85	13 (10–15)	**<0.001**
	Medical/dental	713	116	16 (14–19)	
	Nursing/ midwifery	1,891	395	21 (19–23)	
	Allied health	1,044	118	11 (9.4–13)	
	General support	365	81	22 (18–27)	
	Health care assistant	291	93	32 (27–38)	
	Other	105	10	10 (4.7–17)	
Lives with	Alone	464	61	13 (10–17)	**0.008**
	With others	4,610	834	18 (17–19)	
	Missing	11	3	27 (6.0–61)	
Lives with HCW	Yes	1,571	340	22 (20–24)	**<0.001**
	No	3,412	540	16 (16–17)	
	Missing	102	18	18 (11–26)	

*Calculated using the chi-square test. *Statistically significant findings in bold.*

yet fully vaccinated). The median interval between first and second vaccine dose was 21 days (as recommended before 18th January 2021). For those 23 participants, the median number of days between second vaccine dose and PCR positive test was 30 days (IQR 25–50 days). Five (22%) had symptoms at the time of the positive PCR test and 18 (78%) did not have symptoms. Characteristics of participants with breakthrough infection are shown in **Supplementary Table F** in Annex.

Comparison of Crude Seroprevalence From October 2020 to April 2021

In Hospital 1, the seroprevalence significantly increased from 15% in October 2020 to 21% in April 2021 ($p < 0.001$), and

TABLE 2b | Prevalence of SARS-CoV-2 seropositivity by COVID-19 related characteristics, both hospitals, April 2021.

COVID-19 related characteristics		Total	SARS-CoV-2 seropositive participants		
		N	n	% (95% CI)	*p*-value*
Daily contact with COVID-19 patients	Contact with COVID-19 patients	1,136	265	23 (21–25)	**<0.001**
	Contact with patients without COVID-19	2,500	463	19 (17–20)	
	No patient contact	1,449	170	12 (10–14)	
Previous COVID-19 symptoms (ever)	No symptoms	2,913	169	5.8 (5.0–6.7)	**<0.001**
	Had symptoms	2,171	729	34 (32–36)	
	Missing	1	0	–	
Severity of symptoms	No symptoms	2,913	169	5.8 (5.0–6.7)	**<0.001**
	Mild symptoms	1,502	335	22 (20–24)	
	Significant symptoms	621	364	59 (55–63)	
	Severe (hospitalized)	47	30	64 (49–77)	
	Missing	1	0	–	
Previous positive COVID-19 PCR	No	4,273	235	5.5 (4.8–6.2)	**<0.001**
	Yes	813	663	82 (79–84)	
Symptoms at time of previous positive PCR	No	172	87	51 (43–58)	**<0.001**
	Yes	640	576	90.0 (87–92.2)	
Severity of symptoms at time of PCR	No symptoms	172	87	51 (43–58)	**<0.001**
	Mild symptoms	256	226	88 (84–92.0)	
	Significant symptoms	351	320	91.2 (88–93.9)	
	Severe (hospitalized)	32	30	93.8 (79–99.2)	
	Missing	1	0	–	

*Calculated using the chi-square test. Statistically significant findings in bold.

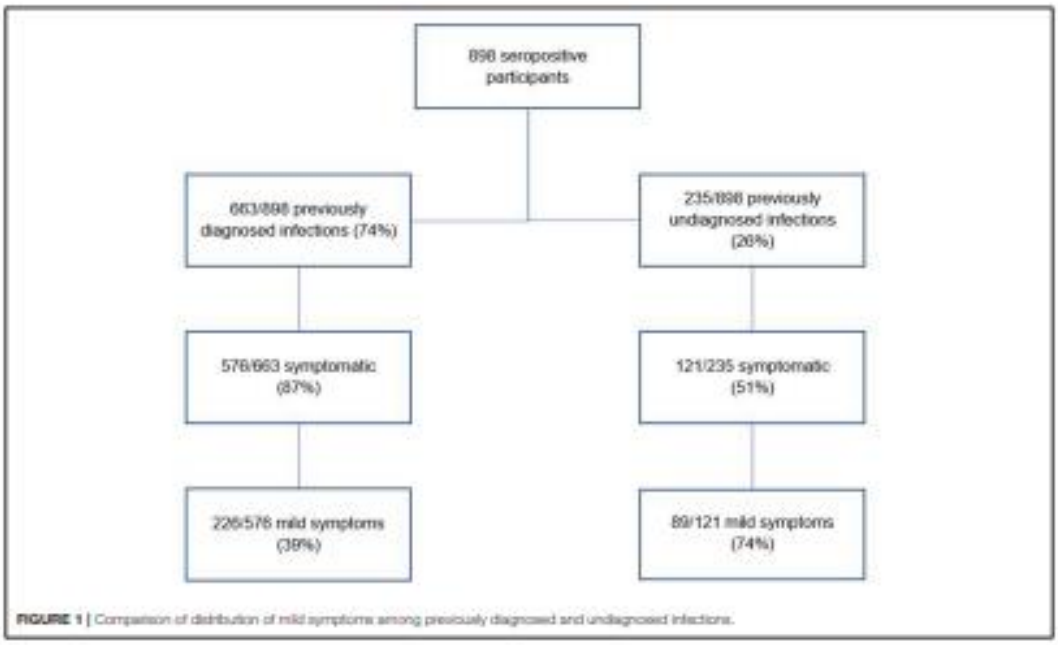


FIGURE 1 | Comparison of distribution of mild symptoms among previously diagnosed and undiagnosed infections.

TABLE 3a | Association between risk factors and SARS-CoV-2 seropositivity, both hospitals, April 2021.

		Unadjusted relative risk (95% CI)	*P*-value risk (95% CI)	Adjusted relative	*P*-value
Hospital	Hospital 2	Ref.			
	Hospital 1	**1.6 (1.4–1.9)**	**<0.001**	**1.5 (1.3–1.8)**	**<0.001**
Age groups (years)	18–29	**1.4 (1.1–1.6)**	**0.001**	**1.3 (1.1–1.6)**	**0.002**
	30–39	1.1 (0.9–1.3)	0.427	1.1 (0.9–1.3)	0.299
	40–49	0.9 (0.7–1.1)	0.209	1.0 (0.7–1.1)	0.569
	50–59	Ref.		Ref.	
	Over 60	1.0 (0.7–1.3)	0.794	1.0 (0.7–1.3)	0.948
Sex	Female	Ref.		Ref.	
	Male	**1.2 (1.1–1.4)**	**0.007**	**1.2 (1.0–1.4)**	**0.016**
Ethnicity	Irish (White)	Ref.		Ref.	
	Any other white background	1.3 (1.0–1.5)	0.020	1.2 (1.0–1.4)	0.120
	Asian background	1.6 (1.3–1.8)	<0.001	1.1 (0.9–1.3)	0.206
	African or other black background	**2.1 (1.6–2.8)**	**<0.001**	**1.7 (1.3–2.2)**	**<0.001**
	Other	1.5 (1.0–2.1)	<0.001	1.3 (0.9–1.9)	0.145
Country of birth	Ireland	Ref.			*
	India	1.7 (1.3–2.0)	<0.001		
	Philippines	1.6 (1.3–2.1)	<0.001		
	United Kingdom	1.0 (0.7–1.3)	0.749		
	Poland	1.5 (1.0–2.2)	0.040		
	USA	1.3 (0.8–2.2)	0.364		
	Romania	2.1 (1.4–3.2)	<0.001		
	Nigeria	3.5 (2.5–4.8)	<0.001		
	Other	1.4 (1.1–1.7)	0.002		
Education	Primary	2.2 (1.2–4.0)	0.014	1.6 (0.9–2.9)	0.138
	Secondary	**1.5 (1.2–1.8)**	**<0.001**	**1.4 (1.1–1.8)**	**0.002**
	Third level	1.3 (1.2–1.5)	<0.001	1.1 (1.0–1.3)	0.133
	Post-graduate	Ref.		Ref.	
Role	Admin	Ref.		Ref.	
	Doctor/Dental	1.3 (1.0–1.7)	0.051	1.0 (0.7–1.4)	0.973
	Nursing	**1.7 (1.3–2.1)**	**<0.001**	**1.4 (1.0–1.8)**	**0.022**
	HCA	**2.5 (2.0–3.3)**	**<0.001**	**1.8 (1.3–2.3)**	**<0.001**
	General support	1.8 (1.3–2.3)	<0.001	1.2 (0.9–1.7)	0.144
	Allied HCW	0.9 (0.7–1.2)	0.424	0.8 (0.6–1.1)	0.119
	Other	0.8 (0.4–1.4)	0.381	0.7 (0.3–1.2)	0.134
Lives with	Alone	Ref.			*
	With others	1.4 (1.1–1.8)	0.010		
Lives with HCW	No	Ref.		Ref.	
	Yes	**1.3 (1.2–1.5)**	**<0.001**	**1.2 (1.0–1.4)**	**0.007**
Workplace exposure to COVID-19 patients	No patient contact	Ref.		Ref.	
	Daily contact with patients without COVID-19	**1.6 (1.3–1.9)**	**<0.001**	**1.3 (1.1–1.5)**	**0.013**
	Daily contact with COVID-19 patients	**2.0 (1.7–2.4)**	**<0.001**	**1.4 (1.1–1.7)**	**0.002**
Previous COVID-19 like symptoms (ever)	No	Ref.			*
	Yes	5.8 (4.9–6.8)	<0.001		
Severity of symptoms	No symptoms	Ref.			*
	Mild symptoms	3.8 (3.2–4.6)	<0.001		
	Significant symptoms	10.1 (8.6–11.9)	<0.001		
	Severe symptoms (hospitalization)	11.0 (8.5–14.3)	<0.001		

Not included in the model. Statistically significant findings in bold.

in Hospital 2 the seroprevalence significantly increased from 4.1% in October 2020 to 13% in April 2021 ($p < 0.001$). For both hospitals combined, seroprevalence increased from 10% in October 2020 to 18% in April 2021. By ethnic group, the increase in seroprevalence was most pronounced for HCWs of African or other black background (from 14% in October 2020 to 33% in April 2021; $p = 0.001$). By education level, increase in seroprevalence was highest for HCWs with lower education levels; the increase for those of primary education (from 14% in October 2020 to 32% in April 2021) was high but not significant due to low numbers in this subgroup ($p = 0.121$); for those of secondary level education the increase was also high (from 8.9 to 22%) and it was significant ($p < 0.001$). By professional subgroup, increase in seroprevalence was highest for general support staff (from 7.6% in October 2020 to 22% in April 2021; $p < 0.001$) and for HCAs (from 18% in October 2020 to 32% in October 2021; $p < 0.001$). A comparison of crude seroprevalence in October 2020 and in April 2021 by participant characteristics and by hospital is shown in **Supplementary Table 4** in Annex.

DISCUSSION

Participation and Demographics

Our participants were similar in age and sex to those in other European studies (7, 29, 31). The participation rate was acceptable for an institutional opt-in study, was comparable to other European studies (32), and included representation from all HCW groups, including the traditionally harder-to-reach groups such as general support staff and healthcare assistants, who may not engage as frequently with hospital communications platforms.

Overall Seroprevalence

The difference in seroprevalence between the two sites primarily reflects the difference in community incidence, with a corresponding higher seroprevalence seen in the more densely populated capital city of Dublin, which has had higher community incidence throughout the pandemic thus far. Other studies have also shown community incidence to be one of the main factors impacting risk to HCW (33, 34). The rise in seroprevalence at both sites reflects the magnitude of the third wave of the pandemic throughout Ireland. The relatively higher increase in seroprevalence in Hospital 2 compared to Hospital 1 likely reflects the fact that the community incidence in the Galway area approached that of the Dublin incidence during this third COVID-19 wave (22).

The difference in overall seroprevalence was also likely to have been impacted by differences in hospital infrastructure, work-practices, bed-flow management, and the differing demographic and social factors by HCW role at each site. Broad work-place practices in both hospitals have been similar throughout the pandemic, including ward-based medical teams and universal use of face masks. There were no issues with personal protective equipment (PPE) availability in either of the hospitals involved in our study at any stage thus far during the pandemic, and where staff were re-deployed to improve the hospital's capacity to deal with the outbreak, staff were not deployed to areas that would have been outside of their scope of practice, and all staff had training on the correct use of PPE. Both hospitals experienced multiple outbreaks during the 3rd wave of the pandemic, however the absence of whole genomic sequencing (WGS) in this study precludes identifying the role of hospital outbreaks in influencing the overall seroprevalence. A recently published Dutch study (conducted prior to HCW vaccination) used WGS to show that transmission to HCW was largely from other HCW—interestingly, they showed a complete absence of transmission from patients to HCW (35). While this may be the case in our cohort, the fact that HCW role and patient proximity were independent risk factors for seropositivity lead us to believe that acquisition by HCW in the hospital setting may have been from both patients and other HCW. It is also difficult to identify the proportion of the risk that is attributable to the workplace vs. the community/ household/ social factors. It is likely that transmission also took place in the household, where higher attack rates are seen (36). It is also unclear if differential timing and spread of VOCs in each location may have impacted overall seroprevalence. Accurate assessment of the risk to HCW in each of these situations and environments, and the impact of VOCs on overall seroprevalence, would necessitate WGS of all HCW with confirmed infection. Real time results would be ideal in order to inform local IPC measures.

The overall seroprevalence in both hospitals was higher than the European average of 8.5% in a recently published meta-analysis (37), however this meta-analysis only took into account studies published up until August 2020. Individual studies across Europe in the first year of the pandemic showed a wide range of SARS-CoV-2 seroprevalence among HCW (1–45%) (7, 31, 38–41). A recently published study of over 80,00 HCW in Italy showed a seroprevalence of 12% amongst HCW, however the serological testing was conducted in April and May 2020 (32). Assumably the seroprevalence amongst HCW has risen across the rest of Europe and worldwide since then, as our study shows it has in Ireland, however, to the best of our knowledge, there are no published studies estimating SARS-CoV-2 seroprevalence in HCW in Europe in 2021, nor comparing this to seroprevalence in 2020.

Seroprevalence by Role and Type of Patient Contact

Daily contact with patients with known/suspected COVID-19 infection was associated with higher seroprevalence, followed by daily contact with patients without known or suspected COVID-19 infection. HCWs with little or no patient contact had the lowest seroprevalence, however we showed in October 2020 that non-patient-facing staff, although less likely to be seropositive, were more likely to have had an undiagnosed infection [i.e., to be antibody positive with no history of PCR-confirmed COVID-19 infection (30)]. This reflects the findings of other studies, including our own findings in these same hospitals in October 2020 (23, 42–44). In terms of working role, being a nurse or a HCA carried a higher aRR, likely also reflecting the close patient contact involved in performing these roles. This finding has also been shown in other large European studies (32).

The seroprevalence among general support staff (which includes domestic and catering staff, maintenance, security and porters) trebled from October 2020 to April 2021. This increase was in both locations and was across all groups in this category (**Supplementary Table 2f** in Annex). This was possibly related to outbreaks amongst these staff groups, though it was not clear whether these outbreaks were related to the workplace or not. There may be improper compliance with use of PPE, and fatigue with other elements of infection prevention and control (IPC) precautions as the pandemic has progressed. There are likely to also be other social factors involved that our study was not designed to assess.

Previous Symptoms and Testing

Nineteen percent of participants with detectable antibodies reported never having experienced symptoms that were consistent with infection with COVID-19. This falls within the broad range reported by other studies (45). Those who had symptomatic infection had a higher rate of antibody positivity than those who had an asymptomatic infection (90 vs. 51%), which is also in keeping with other published data (46). Over a quarter of participants reported having COVID-19 like symptoms at some stage but never having a positive PCR. This highlights the potential overlap in symptoms with other circulating viruses, including rhinoviruses which were circulating widely over the winter of 2020/21 in Ireland (47), and is a reminder of the impossibility of clinically excluding COVID-19 infection in HCW with symptoms, including in those with only mild symptoms, especially over the winter period. It also highlights the complexity involved in developing case definitions and testing guidelines for symptomatic individuals. A subset of these participants never sought testing, despite their symptoms, which reinforces the need for clear messaging about availability of and indication for testing.

Undiagnosed Infections

In both hospitals, the seroprevalence was higher than the known PCR-confirmed diagnoses of COVID-19 infection of the same timeframe (21 vs. 18% in Hospital 1, and 13 vs. 9.2% in Hospital 2) (48). Over a quarter of infections were undiagnosed, and those with an undiagnosed infection were more likely to have been asymptomatic. This proportion of undiagnosed infection had decreased from 39% in October 2020, assumedly due to increased testing and increased awareness, however it remains high despite both hospitals having onsite PCR testing available to HCW with symptoms or those identified as a close contact with a confirmed case of COVID-19 from mid-March 2020. Those with undiagnosed infection fall into two categories; those with asymptomatic infection, and those who did have symptoms but did not seek testing. Most of these undiagnosed infections were associated with none or only mild symptoms, however it is still possible that these undiagnosed HCW were working during the infectious period, with potential for onwards transmission to patients and other staff members if proper use of PPE and other IPC measures were not strictly adhered to. Onwards transmission in the household is even more likely in this setting, where ongoing unprotected close contact is likely. Easy access to testing, early detection of infection, and ongoing adherence to standard infection control precautions at all times, as well as the appropriate use of PPE including face masks in the hospital setting, irrespective of symptoms remain important (49). This finding also supports the recommendation for screening of asymptomatic staff, including vaccinated staff in certain situations, when a hospital outbreak of infection with COVID-19 occurs. The exact role and methodology of routine asymptomatic screening, either widespread or in certain HCW groups, remains to be defined. Regarding those who had symptoms but did not seek testing, concerted efforts need to be made to ensure all HCW groups, including those who are non-patient-facing, are aware of the availability of and need to seek advice regarding testing.

Risk Factors for Antibody Positivity

The main risk factors statistically significantly associated with antibody positivity (in decreasing order of aRR) were being a HCA, being of Black ethnicity, working in Hospital 1, lower level of education, being a nurse, having daily contact with patients (especially those known or suspected to have COVID-19 infection), being age 18–29 years, living with other HCW and being male. Seroprevalence by age and sex were similar to previously published literature (6, 23, 37). Similar findings of increased risk with direct patient contact, the role of HCA, and working with patients with COVID-19 infection have also been reported in the literature (7, 37, 50, 51).

Apart from the changes in seroprevalence by role discussed above, there were two main new findings on multivariable analysis between October 2020 and April 2021. Firstly, lower level of education emerged as an independent risk factor for seropositivity. Lower socio-economic status has been previously noted to correlate with increased risk of COVID-19 infection, and increased risk of poor clinical outcomes (52, 53). The second notable new finding was a change in the seropositivity by ethnic group; the seroprevalence amongst those of Black ethnicity trebled [from 14% (16/113) to 43% (39/117), for an aRR of 1.7 (95% CI 1.3–2.2, $p < 0.001$)]. They were also more likely to be asymptomatic, but not more likely to have an undiagnosed infection. Those of Asian ethnicity had a higher risk of seropositivity in October 2020, but this finding was no longer significant in April 2021. Both of these ethnic groups, as well as other minority ethnic groups which were likely under-represented in our study, have been shown to have increased risk in other studies (37, 54–56). There are likely to be social factors contributing to these ethnicity-related findings in both hospitals that our study did not measure. Ethnicity and level of education have been shown throughout the pandemic to be associated with seropositivity and with poor outcomes of COVID-19 infection. There may be some unmodifiable factors contributing to this risk, however all efforts need to continue to be made to mitigate modifiable risk factors amongst these groups; we cannot assume, even at this stage of the pandemic, that educational messaging regarding risk reduction has reached all groups equally.

Living with other HCW carried an increased risk for seropositivity, similar to our previous findings. This supports the theory that a proportion of the HCW contracting COVID-19 are

doing so in their home environment. This finding was stronger in Hospital 1, where the community incidence was higher and the density of shared living space is also likely to be higher. Other studies have found correlation between size of household and antibody positivity (31) as well as higher risk of COVID-19 with a known household contact (57). To the best of our knowledge ours is the first study to comment on a significant risk of antibody positivity in HCW living with other HCW. This finding was common to both of our serosurveys (23).

Antibody Response to Vaccination

Most participants were either fully or partially vaccinated. All fully vaccinated participants, and the majority of partially vaccinated participants, had detectable anti-S antibodies. Other studies have also shown high rates of seropositivity after both first and second dose vaccination (25, 58, 59). There were less infections in those fully vaccinated than in those partially vaccinated (13 vs. 0.6% of participants reported infection post first and second vaccine dose, respectively), despite the fact that almost all of these participants had detectable antibodies. The 23 breakthrough infections, in 0.6% of the fully vaccinated study population, are in keeping with the rate of breakthrough infections experienced internationally (60). These breakthrough infections serve as a reminder that vaccination does not prevent infection acquisition, even in the setting of confirmed serological response to vaccination. Most of those with breakthrough infections had no symptoms, in keeping with the literature on vaccine effectiveness in reduction of severe symptoms and hospitalization (61, 62) however the numbers are too small for any statistical comparison with symptoms in those who were unvaccinated. It would be prudent for all IPC measures to remain in place in the hospital setting, including for vaccinated HCW, while research is ongoing into the effects of vaccination on infection acquisition and onwards transmission of SARS-CoV-2, including with VOCs. Vaccinated HCW should not be exempt from measures discussed above in relation to minimizing the rate of undiagnosed infections (access to testing, adherence to standard IPC precautions and inclusion in screening of asymptomatic staff in the case of a hospital outbreak).

LIMITATIONS

This study has several limitations. Firstly, information on COVID-19 symptoms and PCR test results were self-reported and thus could be biased and could not be verified. Secondly, although the uptake rate was good for an opt-in study, it was lower than the uptake for PRECISE 1; declining interest in research in the area of COVID-19 is a natural phenomenon as the pandemic progresses. Many staff at this point in time already knew they had been infected and therefore may have less interest in participating and availing of serology testing. Most staff had been vaccinated and therefore may also have a degree of comfort that produces less interest in knowing their antibody status. This could lead to underestimation of the overall seroprevalence. PRECISE 2 took place during the third wave of the pandemic in Ireland, which was the largest in magnitude and the longest. Other limitations include that WGS data were not available, particularly for those infected after full vaccination, and also that information on biological factors, e.g., co-morbidities, was not available. Although the communication strategy was an important part of the recruitment process, the study took part during our third wave of the pandemic, during Level 5 restrictions—the highest level of COVID-19 national restrictions—and therefore also relied heavily on engagement with information technology (IT) platforms (email, messenger groups, hospital intranet) and less on face-to-face announcements. Thirdly, as with all opt-in studies, there may be a selection bias. Those who had been vaccinated may have had less interest in participating due to less curiosity about their own serostatus, and therefore we may have underestimated the overall vaccination status of the workforce (however vaccination coverage amongst our study participants equates to local unpublished hospital data so we feel the study population was representative on this front). Conversely, those who were unvaccinated may have had a fear of having to announce their vaccine-status to the study team, despite results not being linked to occupational health records, and those who had been vaccinated may have been more likely to participate as they may be more likely to have health seeking behavior. The online consent process, questionnaire, and blood test booking system risks exclusion of those who are less literate in IT. This was identified as a potential limitation from the start, and attempts were made to mitigate this selection bias. Multilanguage information and plain English were used, and groups identified as potentially at risk of exclusion on this basis were targeted directly for inclusion in the study, with small-group sessions to aid consent and questionnaire completion and walk-in clinics for phlebotomy. We do not have individual level information on reasons for non-participation, or socio-demographic status of non-participants for comparison, but level of uptake by professional role was deemed to be representative of the hospital HCW population in both hospitals. The absence of genomic sequencing data precludes identifying the role of hospital outbreaks in influencing the overall seroprevalence, as well as drawing any definite conclusions regarding attributable risk to the workplace vs. the community for HCW.

Detection of anti-spike antibody in conjunction with a self-reported history of vaccination was considered to be as a response to vaccination. It is possible that some of these participants may have detectable anti-spike antibody related to natural infection; these were not counted when assessing overall seroprevalence of presumed past infection, and therefore this overall seroprevalence may be an underestimate. Seroprevalence could also be underestimated because recent infection could be missed as several days are required for seroconversion of SARS-CoV-2 infection. Finally, some false negatives and false positives are expected with all laboratory tests. When the estimate of seroprevalence is adjusted using the manufacturer's stated specificity of 99.8% and sensitivity of 99.5%, the seroprevalence does not change (18%; 95% CI 17–19%).

CONCLUSION AND RECOMMENDATIONS

To the best of our knowledge there is no other published study to date estimating SARS-CoV-2 seroprevalence in a large HCW group in 2021. This study is a unique comparison between

two hospitals in areas of differing community incidence at two points in time. The rise in seroprevalence between October 2020 and April 2021 reflects the magnitude of the third wave of the pandemic in both locations. Many of the risk factors identified point toward hospital acquisition of infection (HCW role and type of patient contact) while others suggest that infection was acquired in the household (living with others, living with other HCW); genomic sequencing is needed to identify the role of hospital outbreaks in influencing the overall seroprevalence among HCW, as well as to draw conclusions regarding attributable risk to the healthcare environment vs. the community or household.

Our study highlights the changing epidemiology with different waves of the pandemic, and in different locations. More serological studies are needed in HCW worldwide to assess these changes in risk factors as the pandemic progresses. While each wave of the pandemic may be associated with different risk factors in different locations, many risk factors also remain constant as the pandemic continues worldwide, such as the association of seropositivity with lower level of education and ethnicity; the ongoing presence of these risk factors a year and a half into the pandemic may indicate that some groups have not been adequately reached, despite widespread messaging and education. Concerted efforts are needed to specifically mitigate modifiable risk factors such as ethnicity and level of education.

The antibody response to vaccination is reassuring however further studies are needed to correlate serological response with functional immunity and to estimate duration of protection from infection, particularly with the ongoing emergence of variants of concern. With emerging evidence of reduction in transmission from vaccinated individuals, the authors strongly endorse immediate vaccination of all HCW who have not yet been vaccinated. Our study did show confirmed infection in a small minority of fully vaccinated participants, all with appropriate antibody response to the vaccine, therefore messaging to HCW regarding the role and limits of vaccination need to be clear and should include the ongoing risk of infection and transmission.

The proportion of infections that were previously undiagnosed decreased from October 2020 to April 2021 but remained high. In light of both the undiagnosed infections and the breakthrough infections in vaccinated individuals, ongoing adherence to all infection prevention and control standards in the healthcare setting and household are paramount. It is essential that HCW have easy access to testing, even with mild or no symptoms, and even in the post-vaccination setting (63). We recommend ongoing risk assessment in the setting of a hospital outbreak, and where indicated, screening of HCW, including those without symptoms, and including those who are vaccinated. Breakthrough infections will continue to occur and need to be further characterized; HCW are an ideally positioned group for this due to their increased exposure, and in the interim, we should not change established infection prevention and control measures or testing protocols amongst HCW on the basis of vaccination alone. Assessment of vaccinated HCW with PCR-confirmed SARS-CoV-2 infection should include SARS-CoV-2 WGS and, if possible, SARS-CoV-2 WGS of index cases identified by follow-up, as well as assessment of potential host determinants of infection, in order to advance understanding of risk factors for breakthrough infection.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by National Research Ethics Committee (NREC) - Ireland. The patients/participants provided their written informed consent to participate in this study.

PRECISE STUDY (PREVALENCE OF COVID-19 IN IRISH HEALTHCARE WORKERS) STEERING GROUP MEMBERS AND AFFILIATIONS

Dr. Loraine Doherty, National Clinical Director for Health Protection, HSE-Health Protection Surveillance Centre (HPSC), Dublin, Ireland, and Chair of Steering Group, Dr. Niamh Allen, Consultant Physician in Infectious Diseases and Principal Investigator for PRECISE study, Professor Colm Bergin, Consultant Physician in Infectious Diseases and Site Lead for PRECISE study, St. James's Hospital Dublin, Ireland, Dr. Niall Conlon, Consultant Immunologist, St. James's Hospital, Dublin, Ireland, Dr. Lisa Domegan, Surveillance Scientist, HSE-HPSC, Dublin, Ireland, Dr. Catherine Fleming, Consultant in Infectious Disease and Site Lead for PRECISE study, Galway University Hospital, Galway, Ireland, Dr. Margaret Fitzgerald, National Public Health Lead. National Social Inclusion Office, Dublin, Ireland, Dr. Cillian de Gascun, Director, UCD National Virus Reference Laboratory, University College Dublin, Dublin, Ireland, Joan Gallagher, Programme Manager, Office of the National Clinical Director for Health Protection, HSE HPSC, Dublin, Ireland, Dr. Derval Igoe, Specialist in Public Health Medicine, HSE HPSC, Dublin, 1, Ireland, Prof. Mary Keogan, Consultant Immunologist Beaumont Hospital & Clinical Lead, National Clinical Programme for Pathology, HSE, Ireland, Dr. Noirin Noonan, Consultant in Occupation Medicine, St. James's Hospital, Dublin, Ireland., Professor Cliona O'Farrelly, Chair in Comparative Immunology, Trinity College Dublin, Ireland, Dr. Una Ni Riain, Consultant Microbiologist, Galway University Hospital, Galway, Ireland, Dr. Breda Smyth, Department of Public Health, HSE West, Ireland.

AUTHOR CONTRIBUTIONS

NA was the principal investigator for the study, designed and executed the study in collaboration with the lead site investigators

and the Study steering group, organized the data collection, aided interpretation of the data, and wrote the scientific manuscript. MB analyzed the data with the support of AC, LiD, and CW. CB and CF were the lead site investigators and aided with the design and execution of all aspects of the study. NC and UN coordinated the laboratory testing of all samples. LoD was the study coordinator and oversaw all aspects of the running of the study, and coordinated the PRECISE Study Steering Group, who aided with study design and execution. EH and CK supported data collection. All authors reviewed the final manuscript.

FUNDING

This work was supported financially by the Irish Health Service Executive COVID-19 budget. The funders had no role in the design of the study, the data analysis, or the decision to publish. The funders are providing funding for open access publication fees.

ACKNOWLEDGMENTS

We would like to acknowledge the National Public Health Emergency Team (NPHET) who supported this study, the study steering group who planned the study and critically evaluated this manuscript, the study team who coordinated the running of the study in each hospital, the hospital management at both sites for their support for the study, and the staff of both hospitals who participated. We would especially like to acknowledge the phlebotomy departments in each hospital for facilitating the sampling of over 5,000 participants, the virology and biochemistry laboratories in each hospital for processing the samples and the human resources department for their help with denominator data.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.758118/full#supplementary-material

REFERENCES

1. Shah ASV, Wood R, Gribben C, Caldwell D, Bishop J, Weir A, et al. Risk of hospital admission with coronavirus disease 2019 in healthcare workers and their households: nationwide linkage cohort study. *BMJ*. (2020) 371:m3582. doi: 10.1136/bmj.m3582
2. Karlsson U, Frankel C-J. COVID-19: risks to healthcare workers and their families. *BMJ*. (2020) 371:m3944. doi: 10.1136/bmj.m3944
3. Richterman A, Meyerowitz EA, Cevik M. Hospital-acquired SARS-CoV-2 infection: lessons for public health. *JAMA*. (2020) 324:2155. doi: 10.1001/jama.2020.21399
4. Watson J, Richter A, Deeks J. Testing for SARS-CoV-2 antibodies. *BMJ*. (2020) 370:m3325. doi: 10.1136/bmj.m3325
5. *Preliminary Report of the Results of the Study to Investigate COVID-19 Infection in People Living in Ireland (SCOPI): A National Seroprevalence Study*. (2020). Available online at: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/scopi/SCOPI%20report%20preliminary%20result%20final%20version.pdf (accessed August 10, 2021).
6. Allen N. *Prevalence of Antibodies to SARS-CoV-2 in Irish Healthcare Workers Phase 1*. (2020). Available online at: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/research/precise/PRECISE%20Study%20Phase%201%20Interim%20Report%20January%202021.pdf (accessed August 10, 2021).
7. Psichogiou M, Karabinis A, Poulopoulou ID, Basoulis D, Petsios K, Roussos S, et al. Antibodies against SARS-CoV-2 among health care workers in a country with low burden of COVID-19. *PLoS ONE*. (2020) 15:e0243025. doi: 10.1371/journal.pone.0243025
8. Hanrath AT, Payne BAI, Duncan CJA. Prior SARS-CoV-2 infection is associated with protection against symptomatic reinfection. *J Infect*. (2020) 82:e29–30. doi: 10.1016/j.jinf.2020.12.023
9. *Health Information and Quality Authority: Advice to the National Public Health Emergency Team; Duration of Immunity Following SARS-CoV-2 Infection*. (2021). Available online at: https://www.hiqa.ie/sites/default/files/2021-03/Duration-of-protective-immunity_Advice-to-NPHET-24%20-Feb-2021.pdf (accessed August 10, 2021).
10. Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet*. (2021) 397:99–111. doi: 10.1016/S0140-6736(20)32661-1
11. Thompson MG, Burgess JL, Naleway AL, Tyner H, Yoon SK, Meece J, et al. Prevention and attenuation of COVID-19 with the BNT162b2 and mRNA-1273 vaccines. *N Engl J Med*. (2021) 385:320–9. doi: 10.1056/NEJMoa2107058
12. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N Engl J Med*. (2021) 384:403–16. doi: 10.1056/NEJMoa2035389
13. Jara A, Undurraga EA, González C, Paredes F, Fontecilla T, Jara G, et al. Effectiveness of an inactivated SARS-CoV-2 vaccine in Chile. *N Engl J Med*. (2021) 385:875–84. doi: 10.1056/NEJMoa2107715
14. Sahin U, Muik A, Derhovanessian E, Vogler I, Kranz LM, Vormehr M, et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. *Nature*. (2020) 586:594–9. doi: 10.1038/s41586-020-2814-7
15. Lumley SF, O'Donnell D, Stoesser NE, Matthews PC, Howarth A, Hatch SB, et al. Antibody status and incidence of SARS-CoV-2 infection in health care workers. *N Engl J Med*. (2021) 384:533–40. doi: 10.1056/NEJMoa2034545
16. Meyer B, Drosten C, Müller MA. Serological assays for emerging coronaviruses: challenges and pitfalls. *Virus Res*. (2014) 194:175–83. doi: 10.1016/j.virusres.2014.03.018
17. Okba NMA, Müller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease patients. *Emerg Infect Dis*. (2020) 26:1478–88. doi: 10.3201/eid2607.200841
18. Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet*. (2020) 396:467–78. doi: 10.1016/S0140-6736(20)31604-4
19. Walsh EE, Frenck RW, Falsey AR, Kitchin N, Absalon J, Gurtman A, et al. Safety and Immunogenicity of Two RNA-based COVID-19 vaccine candidates. *N Engl J Med*. (2020) 383:2439–50. doi: 10.1056/NEJMoa2027906
20. Dörschug A, Frickmann H, Schwanbeck J, Yilmaz E, Moter K, Hahn A, et al. Comparative assessment of sera from individuals after S-gene RNA-based SARS-CoV-2 vaccination with spike-protein-based and nucleocapsid-based serological assays. *Diagnostics*. (2021) 11:426. doi: 10.3390/diagnostics11030426
21. *HPSC COVID Cases in Ireland*. Available from: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/casesinireland/epidemiologyofcovid-19inireland/COVID19%20Daily%20Infographic.pdf (accessed May 12, 2021).

22. *Epidemiology of COVID-19 in Ireland - 14 day Report.* Report prepared by HPSC on 24/01/2021. Available online at: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/surveillance/covid-19infectionepidemiologyreports/2021/january2021/COVID-19_14_day_epidemiology_report_20210124_Website.pdf (accessed Apr 19, 2021).
23. Allen N, Ní Riain U, Conlon N, Ferenczi A, Carrion Martin AI, Donnagan L, et al. Prevalence of antibodies to SARS-CoV-2 in Irish hospital healthcare workers. *Epidemiol Infect.* (2021) 149:e157. doi: 10.1017/S0950268821000984
24. Castor EDC. *Castor Electronic Data Capture.* (2021). Available online at: https://castoredc.com; https://www.castoredc.com/
25. CDC *Real-World Study Confirms Protective Benefits of mRNA COVID-19 Vaccines.* (2021). Available online at: https://www.cdc.gov/media/releases/2021/p0329-COVID-19-Vaccines.html (accessed Jun 16, 2021).
26. *National Immunisation Advisory Committee (NIAC) Guidelines for Immunisation Chapter 5A- COVID-19.* (2021). Available online at: https://www.hse.ie/eng/health/immunisation/hcpinfo/guidelines/ (accessed Jun 18, 2021).
27. *Elecsys® Anti-SARS-CoV-2 Immunoassay for the qualitative detection of antibodies (incl. IgG) against SARS-CoV-2.* Available online at: https://diagnostics.roche.com/global/en/products/params/elecsys-anti-sars-cov-2.html (accessed Dec 14, 2020).
28. *Elecsys® Anti-SARS-CoV-2 S Immunoassay for the Quantitative Determination of Antibodies to the SARS-CoV-2 Spike Protein.* Available online at: https://diagnostics.roche.com/global/en/products/params/elecsys-anti-sars-cov-2-s.html (accessed May 20, 2021).
29. Plebani M, Padoan A, Fedeli U, Schievano E, Vecchiato E, Lippi G, et al. *SARS-CoV-2 Serosurvey in Health Care Workers of the Veneto Region.* Clinical Chemistry and Laboratory Medicine (CCLM) (2020). Available online at: https://www.degruyter.com/view/journals/cclm/ahead-of-print/article-10.1515-cclm-2020-1236/article-10.1515-cclm-2020-1236.xml (accessed Dec 14, 2020).
30. *Prevalence of Antibodies to SARS-CoV-2 Natural Infection and Post-vaccination in Irish Hospital Healthcare Workers (PRECISE 2).* Health Protection Surveillance Centre of Ireland (HPSC) (2021) Available online at: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/research/precise/PRECISE%202%20Report.pdf (accessed Jul 26, 2021).
31. García-Basteiro AL, Moncunill G, Tortajada M, Vidal M, Guinovart C, Jiménez A, et al. Seroprevalence of antibodies against SARS-CoV-2 among health care workers in a large Spanish reference hospital. *Nat Commun.* (2020) 11:3500. doi: 10.1038/s41467-020-17318-x
32. Poletti P, Tirani M, Cereda D, Gazzetta G, Trentini F, Marziano V, et al. Seroprevalence of and risk factors associated with SARS-CoV-2 infection in health care workers during the early COVID-19 pandemic in Italy. *JAMA Netw Open.* (2021) 4:e2115699. doi: 10.1001/jamanetworkopen.2021.15699
33. Jacob JT, Baker JM, Fridkin SK, Lopman BA, Steinberg JP, Christenson RH, et al. Risk factors associated with SARS-CoV-2 seropositivity among US health care personnel. *JAMA Netw Open.* (2021) 4:e211283. doi: 10.1001/jamanetworkopen.2021.1283
34. Rahnke GW, Richterman A. Predictors of COVID-19 seropositivity among healthcare workers: an important piece of an incomplete puzzle. *J Hosp Med.* (2021) 16:320.doi: 10.12788/jhm.3632
35. Sikkema JJ, Bain DTP, Peters EJG, Dekker M, Schinkel M, Reijnders TDY, et al. Serologic surveillance and phylogenetic analysis of SARS-CoV-2 infection among hospital health care workers. *JAMA Netw Open.* (2021) 4:e2118554. doi: 10.1001/jamanetworkopen.2021.18554
36. Madewell ZJ, Yang Y, Longini IM, Halloran ME, Dean NE. Household transmission of SARS-CoV-2: a systematic review and meta-analysis. *JAMA Netw Open.* (2020) 3:e2031756. doi: 10.1001/jamanetworkopen.2020.31756
37. Galanis P, Vraka I, Fragkou D, Bilali A, Kaitelidou D. Seroprevalence of SARS-CoV-2 antibodies and associated factors in health care workers: a systematic review and meta-analysis. *J Hosp Infect.* (2021) 108:120–34. doi: 10.1101/2020.10.23.20218289
38. Korth J, Wilde B, Dolff S, Anastasiou OE, Krawczyk A, Jahn M, et al. SARS-CoV-2-specific antibody detection in healthcare workers in Germany with direct contact to COVID-19 patients. *J Clin Virol.* (2020) 128:104437. doi: 10.1016/j.jcv.2020.104437
39. Jespersen S, Mikkelsen S, Greve T, Kaspersen KA, Tolstrup M, Boldsen JK, et al. SARS-CoV-2 seroprevalence survey among 17,871 healthcare and administrative personnel at hospitals, pre-hospital services, and specialist practitioners in the Central Denmark Region. *Clin Infect Dis.* (2021) 73:e2853–60. doi: 10.1093/cid/ciaa1471
40. Steensels D, Oris E, Coninx L, Nuyens D, Delforge M-L, Vermaercke P, et al. Hospital-wide SARS-CoV-2 antibody screening in 3056 staff in a tertiary center in Belgium. *JAMA.* (2020) 324:195. doi: 10.1001/jama.2020.11160
41. Houlihan C, Vora N, Byrne T, Lewer D, Heaney J, Moore DA, et al. SARS-CoV-2 virus and antibodies in front-line Health Care Workers in an acute hospital in London: preliminary results from a longitudinal study. *Infect Dis.* (2020). doi: 10.1101/2020.06.08.20126904
42. Kahlert CR, Persi R, Güsewell S, Egger T, Leal-Neto OB, Sumer J, et al. Non-occupational and occupational factors associated with specific SARS-CoV-2 antibodies among hospital workers – a multicentre cross-sectional study. *Clin Microbiol Infect.* (2021) 27:1336–44. doi: 10.1016/j.cmi.2021.05.014
43. Galanis P, Vraka I, Fragkou D, Bilali A, Kaitelidou D. Seroprevalence of SARS-CoV-2 antibodies and associated factors in healthcare workers: a systematic review and meta-analysis. *J Hosp Infect.* (2021) 108:120–34. doi: 10.1016/j.jhin.2020.11.008
44. Ellström KM, Blomqvist J, Nilsson P, Hober S, Piu E, Månberg A, et al. Differences in risk for SARS-CoV-2 infection among healthcare workers. *Prev Med Rep.* (2021) 24:101518. doi: 10.1101/2021.03.30.21254653
45. Gao Z, Xu Y, Sun C, Wang X, Guo Y, Qiu S, et al. A systematic review of asymptomatic infections with COVID-19. *J Microbiol Immunol Infect.* (2021) 54:12–6. doi: 10.1016/j.jmii.2020.05.001
46. Long Q-X, Tang X-J, Shi Q-L, Li Q, Deng H-J, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med.* (2020) 26:1200–4. doi: 10.1038/s41591-020-0965-6
47. *Influenza Surveillance in Ireland – Weekly Report, Influenza Week 13 & 14 (2021) (29th March– 11th April 2021).* Available online at: https://www.hpsc.ie/a-z/respiratory/influenza/seasonalinfluenza/surveillance/influenzasurveillancereports/20202021season/Influenza_Surveillance_Report_Week%2013%20and%2014%202021_Final_1.0V.pdf (accessed August 10, 2021).
48. Local Occupational Health Department databases, Galway University Hospital and St. James's Hospital (2021). Unpublished data.
49. *HPSC Infection Prevention and Control Precautions for COVID-19.* Available online at: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/guidance/infectionpreventionandcontrolguidance/ (accessed Jan 12, 2021).
50. Martin CA, Patel P, Goss C, Jenkins DR, Price A, Barton L, et al. Demographic and occupational determinants of anti-SARS-CoV-2 IgG seropositivity in hospital staff. *J Public Health.* (2020) fdaa199. doi: 10.1093/pubmed/fdaa199. [Epub ahead of print].
51. Lai X, Wang M, Qin C, Tan L, Ran L, Chen D, et al. Coronavirus Disease 2019(COVID-2019) infection among health care workers and implications for prevention measures in a tertiary hospital in Wuhan, China. *JAMA Network Open.* (2020) 3:e209666. doi: 10.1001/jamanetworkopen.2020.9666
52. Hawkins RB, Charles EJ, Mehaffey JH. Socio-economic status and COVID-19–related cases and fatalities. *Public Health.* (2020) 189:129–34. doi: 10.1016/j.puhe.2020.09.016
53. Truong N, Asare AO. Assessing the effect of socio-economic features of low-income communities and COVID-19 related cases: an empirical study of New York City. *Glob Public Health.* (2021) 16:1–16. doi: 10.1080/17441692.2020.1850838
54. Valdes AM, Moon JC, Vijay A, Chaturvedi N, Noerish A, Ikram A, et al. Longitudinal assessment of symptoms and risk of SARS-CoV-2 infection in healthcare workers across 5 hospitals to understand ethnic differences in infection risk. *EClinicalMedicine.* (2021) 34:100835. doi: 10.1016/j.eclinm.2021.100835
55. Aldridge RW, Lewer D, Katikireddi SV, Mathur R, Pathak N, Burns R, et al. Black, Asian and Minority Ethnic groups in England are at increased risk of death from COVID-19: indirect standardisation of NHS mortality data. *Wellcome Open Res.* (2020) 5:88. doi: 10.12688/wellcomeopenres.15922.2
56. Martyn EM, Whitaker H, Gil E, Ighenemho F, Lambe G, Conley R, et al. Disproportionate impact of SARS-CoV-2 on ethnic minority and frontline

healthcare workers: a cross-sectional seroprevalence survey at a North London hospital. *J Infect.* (2021) 82:276–316. doi: 10.1016/j.jinf.2021.02.027

57. Kumar N, Bhartiya S, Desai S, Mutha A, Baldar A, Singh T. Seroprevalence of antibodies against SARS-CoV-2 among health care workers in Mumbai, India. *Asia Pac J Public Health.* (2020) 33:126–8. doi: 10.1177/1010539520977307
58. Eyre DW, Lumley SF, Wei J, Cox S, James T, Justice A, et al. Quantitative SARS-CoV-2 anti-spike responses to Pfizer-BioNTech and Oxford-AstraZeneca vaccines by previous infection status. *Clin Microbiol Infect.* (2021) 27:1516.e7–14. doi: 10.1101/2021.03.21.21254061
59. CDC *Morbidity and Mortality Weekly Report (MMWR) - Interim Estimates of Vaccine Effectiveness of BNT162b2 and mRNA-1273 COVID-19 Vaccines in Preventing SARS-CoV-2 Infection Among Health Care Personnel, First Responders, and Other Essential and Frontline Workers — Eight U.S. Locations, December 2020–March 2021.* (2021) Available online at: https://www.cdc.gov/mmwr/volumes/70/wr/mm7013e3.htm (accessed Jun 16, 2021).
60. CDC COVID-19 Vaccine Breakthrough Case Investigations Team, CDC COVID-19 Vaccine Breakthrough Case Investigations Team, Birhane M, Bressler S, Chang G, Clark T, et al. COVID-19 Vaccine Breakthrough Infections Reported to CDC — United States, January 1–April 30, 2021. *MMWR Morb Mortal Wkly Rep.* (2021) 70:792–3. doi: 10.15585/mmwr.mm7021e3
61. Bernal JL, Andrews N, Gower C, Stowe J, Robertson C, Tessier E, et al. Early effectiveness of COVID-19 vaccination with BNT162b2 mRNA vaccine and ChAdOx1 adenovirus vector vaccine on symptomatic disease, hospitalisations and mortality in older adults in England. *BMJ.* (2021) 373:n1088. doi: 10.1101/2021.03.01.21252652
62. Pritchard E, Matthews PC, Stoesser N, Eyre DW, Gethings O, Vihta K-D, et al. Impact of vaccination on new SARS-CoV-2 infections in the United Kingdom. *Nat Med.* (2021) 27:1370–8. doi: 10.1038/s41591-021-01410-w
63. Brown CS, Clare K, Chand M, Andrews J, Auckland C, Bashir S, et al. Snapshot PCR surveillance for SARS-CoV-2 in hospital staff in England. *J Infect.* (2020) 81:427–34. doi: 10.1016/j.jinf.2020.06.069

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix 2.5 Kerr C, Allen N et al. *Irish Journal of Medical Science* (2021)

Irish Journal of Medical Science (1971 -) (2022) 191:1089–1092
https://doi.org/10.1007/s11845-021-02700-5

BRIEF REPORT

SARS-CoV-2 anti-nucleocapsid assay performance in healthcare workers at baseline and 6 months

Colm Kerr[1,2] · Niamh Allen[1] · Gerry Hughes[1] · Martina Kelly[3] · Fiona O'Rourke[3] · Yvonne Lynagh[3] · Jean Dunne[4] · Brendan Crowley[3] · Niall Conlon[4] · Colm Bergin[1,2]

Received: 1 June 2021 / Accepted: 25 June 2021 / Published online: 7 July 2021

Abstract

Introduction Serological SARS-CoV-2 assays have an important role in guiding the pandemic response. This research aimed to compare the performance of 2 antinucleocapsid assays.

Methods Serum from 49 HCWs was analysed at baseline and 6 months using the Abbott diagnostics SARS-CoV-2 IgG assay and the Roche Diagnostics Elecsys Anti-SARS-CoV-2 total antibody assay.

Results At baseline, 14/49 participants (29%) demonstrated antibody reactivity using the Abbott assay. At 6 months, 4/14 participants (29%) continued to demonstrate reactivity. A total of 14/49 (29%) participants had detectable antibodies at baseline using the Roche assay. In total, 13/14 (93%) of participants demonstrated antibody reactivity at 6 months. The Abbott assay showed a statistically significant difference in the signal-to-threshold values of baseline reactive samples when repeated at 6 months ($p = 0.001$). This was not seen with the Roche assay ($p = 0.51$).

Conclusion In this small study, the Roche Diagnostics Elecsys Anti-SARS-CoV-2 total antibody assay appears superior in performance to the Abbott diagnostics SARS-CoV-2 IgG assay in accurately detecting participants with a history of confirmed COVID-19 disease at 6 months follow-up. This finding should be born in mind in the planning of future seroprevalence studies, especially when considering the use of anti-nucleocapsid assays.

Keywords Antibody · Anti-nucleocapsid · Assay · COVID-19 · SARS-CoV-2

Introduction

Significant interest has surrounded the accuracy and utility of assays for the detection of SARS-CoV-2 antibodies since the emergence of COVID-19 in late 2019, and substantial resources have been allocated to developing and validating these assays since the disease was declared a global pandemic in early 2020.

Such assays have several important roles to play in our response to the pandemic. Epidemiological studies such as SCOPI (Study to Investigate COVID-19 Infection in People Living in Ireland) [1], national programme assessing seropositivity in community-based healthcare workers [2] or more localised studies focusing on specific hospitals such as the PRECISE study (Prevalence of antibodies to COVID-19 in Irish Healthcare workers) [3] can help us better understand disease spread and exposure at overall population as well as at national and local healthcare worker levels. Assays will continue to be important in assessing host vaccine response in trial participants [4] and potentially to inform individual risk of disease [5] with significant implications for healthcare workforce planning, though further research on this area of post-infection immunity is required.

This research compares the baseline SARS-CoV-2 antibody response and antibody persistence at 6 months in healthcare workers using two anti-nucleocapsid assays.

✉ Colm Kerr
colmkerr@gmail.com

[1] Department of Infectious Diseases, St. James's Hospital, Dublin, Ireland

[2] Department of Clinical Medicine, St. James's Hospital, Trinity College Dublin, Dublin, Ireland

[3] Department of Clinical Microbiology, St. James's Hospital, Dublin, Ireland

[4] Department of Immunology, St. James's Hospital, Dublin, Ireland

Methods

Ethical approval for this study was granted by the St. James's Hospital and Tallaght University Hospital research ethics committee in April 2020 (reference 2020–04 List 15).

A validation study in a convenience sample of 49 healthcare workers at St. James's Hospital, Dublin, was commenced in April 2020. Serum samples were tested for SARS-CoV-2 antibodies using two laboratory validated assays, the Roche Diagnostics Elecsys Anti-SARS-CoV2 total antibody assay and the Abbott diagnostics SARS-CoV-2 IgG assay. Testing was repeated in October 2020.

Assay results were interpreted using the manufacturers' recommended cut-off index (COI) values (Abbott COI ≥ 1.40 = reactive, Roche COI ≥ 1.0 = reactive), with values below this COI being deemed non-reactive. In October 2020, Abbott updated their SARS-CoV-2 IgG assay guidance (Abbott Diagnostics Product Information Letter PI1060-2020) to include an optional editable "greyzone" with a COI of 0.5–1.39 which they advised "must be interpreted by the clinician in the context of relevant clinical and laboratory information on the patient".

Data were analysed using MS Excel 2016 and Stata v14. The Wilcoxon signed rank test was used to examine the difference between the baseline and 6-month signal-to-threshold values.

Results

A total of 49 healthcare workers (32 female and 17 male) participated in the study. There were three cohorts of participants (Table 1): those who had (1) experienced symptoms and had COVID-19 disease confirmed by RT-PCR for SARS-CoV-2 ($n = 14$), (2) those who experienced symptoms but were RT-PCR not-detected for SARS-CoV-2 ($n = 15$) and (3) those who were asymptomatic and did not undergo RT-PCR testing ($n = 20$).

Table 2 highlights a marked difference in the performance of the Abbott, and Roche assays can be seen in the signal-to-threshold values at baseline and 6 months among the RT-PCR-detected participants. The average signal-to-threshold values using the Abbott assay (COI ≥ 1.40 = reactive, 0.5 – 1.39 = "greyzone") in this cohort was 4.7. At 6 months, the average value had fallen to 1.1 ($p = 0.001$). This contrasts with the Roche assay (COI ≥ 1.0 = reactive) where the average value at baseline was 31.6 and remained above the COI 6 months later at 50.8 ($p = 0.43$). There were no statistical differences in the signal-to-threshold values at baseline and 6 months for either assay in the RT-PCR not-detected cohort or the asymptomatic cohort.

Thirteen participants returned reactive tests at baseline on both Abbott and Roche assays (supplementary material). Fourteen participants demonstrated reactivity at baseline using the Abbott assay (12 from the RT-PCR detected group, 1 from the RT-PCR detected group and 1 from the asymptomatic group — this patient had recently returned from an area of high SARS-CoV-2 prevalence). Four of these continued to exhibit reactivity at 6 months (all from the RT-PCR-detected group). There were 3 "greyzone" results at baseline, two from the RT-PCR detected group and one from the RT-PCR not-detected group. At 6 months, there were 7 "greyzone" results, 3 from the RT-PCR detected group (all of whom were reactive at baseline), 1 from the RT-PCR not-detected group (the same participant as baseline) and 3 from the asymptomatic group (none of whom were deemed to be reactive or in the "greyzone" at baseline). One of these asymptomatic patients exhibited a reactive result at 6 months using the Roche assay, likely representing interim acquisition.

Fourteen participants in total exhibited reactivity using the Roche assay at baseline (13 from the RT-PCR detected group and 1 from the RT-PCR not-detected group). Thirteen continued to demonstrate reactivity using this assay at 6 months (12 from the RT-PCR detected group and the same participant from the RT-PCR not-detected group), along with 2 new participants (one from the RT-PCR detected group and one from the asymptomatic group who was thought to have had experienced asymptomatic infection) (this participant exhibited a "greyzone" result at 6 months on the Abbott assay as mentioned above).

Figure 1 highlights the signal-to-threshold trends of participants with reactive assay results at baseline. Fourteen

Table 1 Basic demographics of participants

Column	Symptomatic and confirmed COVID-19 by PCR	Symptomatic but COVID-19 not detected on RT-PCR	Asymptomatic
Male	3	4	10
Female	11	16	5
Median age (IQR)	43 yrs (37–52 yrs)	46 yrs (39–51 yrs)	34 yrs (32–38 yrs)
Hospitalised	0	0	N/A
Average day post-symptom onset (range)	30 d (21–36 d)	32 d (22–63 d)	N/A

Table 2 Signal-to-threshold values of assays in all cohorts at baseline and 6 months

	1. Confirmed COVID-19 disease SARS-CoV-2 detected by RT-PCR	2. SARS-CoV-2 not-detected by RT-PCR	3. RT-PCR not tested
	Symptomatic pre baseline		Asymptomatic pre baseline
Abbott diagnostics SARS-CoV-2 IgG assay (COI ≥ 1.40 = reactive, 0.5–1.39 = "greyzone")			
Reactive at baseline	12	1	1
"Greyzone" at baseline	2	1	0
Non-reactive at baseline	0	18	14
Baseline mean signal-to-threshold value (standard deviation)	4.7 (2.4)	0.3 (1.3)	0.1 (0.4)
Reactive at 6 months	4	0	0
"Greyzone" at 6 months	3	1	3
Non-reactive at 6 months	7	19	12
6-month mean signal-to-threshold value (standard deviation)	1.1 (1.2)	0.07 (0.14)	0.17 (0.27)
p-value	$p = 0.001$	$p = 0.20$	$p = 0.28$
Roche Diagnostics Elecsys Anti-SARS-CoV-2 total antibody assay (COI ≥ 1.0 = reactive)			
Reactive at baseline	13	1	0
Non-reactive at baseline	1	19	15
Baseline mean signal-to-threshold value (standard deviation)	31.6 (31)	1.2 (4.6)	0.7 (0.2)
Reactive at 6 months	13	1	1
Non-reactive at 6 months	1	19	14
6-month mean signal-to-threshold value (standard deviation)	50.8 (70.2)	0.6 (2.1)	2.9 (10.8)
p-value	$p = 0.43$	$p = 0.71$	$p = 0.41$

participants demonstrated baseline reactivity using the Abbott assay. Of these 14 participants, only 4 (29%) remained reactive 6 months later with a further 3 participant samples (21%) being in the "greyzone". All of these 7 participants belonged to the RT-PCR detected group. There was a statistically significant difference ($p = 0.001$) in the signal-to-threshold values at baseline (average 5.1) and 6 months later (average 1.1).

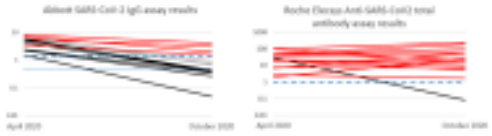


Fig. 1 This figure highlights the trend in assay signal-to-threshold values of participants with reactive assay results at baseline. The red lines represent participants whose samples remained reactive at 6 months; the black lines represent samples that went from being reactive to non-reactive at 6 months. The grey lines represent the participants who were in the "greyzone" at 6 months (Abbott assay only). The long dashed blue line represents the manufacturers COI for reactivity (1.4 for Abbott and 1.0 for Roche). The short dashed blue line represents the beginning of the manufacturer's updated "greyzone" threshold of 0.5 (Abbott assay only)

Fourteen participants also demonstrated baseline reactivity using the Roche assay. Thirteen (93%) remained reactive at 6 months follow-up (12 from the RT-PCR detected and 1 from the RT-PCR not-detected group — the same participant who also demonstrated reactivity at baseline using the Abbott assay). The average signal-to-threshold value at baseline was 33 and was 51.4 at 6 months ($p = 0.51$).

Discussion

Some publications have reported the decline in antibody titre among patients with COVID-19, including Patel et al., who examined changes in antibody titres over 60 days [6], and the publication from Gudbjartsson et al. [7] analysing changes up to 110 days post onset of symptoms. However, there are few articles in the literature that examine this response at 6 months. Muecksh et al. [8] reported the performance of two platforms that employed SARS-CoV-2 spike based antigens (Diasorin Liaison and Siemens Atellica) and two that employed nucleocapsid based antigens (Abbott Architect and Roche Elecsys). Though this study did not look at assay performance at 6 months as our study has, they did note that the sensitivity of the Abbott Architect declined over time.

Lumley et al. [9] recently examined the performance of an anti-nucleocapsid (Abbott Architect) and an anti-spike IgG ELISA (developed by the University of Oxford [10]) in healthcare workers over 6 months. They found that the anti-nucleocapsid antibodies wane within months and faster in younger adults and those without symptoms but that the anti-spike IgG remains stably detected.

In this small study, a statistically significant drop in the signal-to-threshold values from was seen in the 6-month follow-up antibody results of baseline seropositive participants and RT-PCR detected participants using the Abbott assay. This finding was not seen with the Roche assay. The reason behind this drop is unclear, but it may be due to the polyvalent nature of the Roche assay.

Conclusion

Our research shows the variable persistence of detectable antibodies among participants at 6 months with two different anti-nucleocapsid assays. The Roche Diagnostics Elecsys Anti-SARS-CoV-2 total antibody assay appears superior in performance to the Abbott diagnostics SARS-CoV-2 IgG assay in correctly identifying participants with prior confirmed COVID-19 disease at 6 months follow-up. This finding should be born in mind in the planning of future seroprevalence studies, especially when considering the use of anti-nucleocapsid assays.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s11845-021-02700-5.

Author contribution CK and NA contributed to planning, conducting, design, data acquisition, analysis of data and writing of the manuscript. YL, MK, FOR and BC contributed to the planning, conducting and data acquisition. GH and JD contributed to planning, conducting, data acquisition and writing of the manuscript. NC and CB contributed to planning, conduction, design and manuscript review.

Funding Open Access funding provided by the IReL Consortium Funding was received from Trinity College Dublin's Covid-19 response funding call.

Availability of data and material Data is available upon request.

Declarations

Ethical approval Ethical approval for this study was granted by the St. James's Hospital and Tallaght University Hospital research ethics committee in April 2020 (reference 2020-04 List 15).

Consent to participate and publication All participants provided informed written consent for their participation and publication of findings.

Conflict of interest The authors declare no competing interests.

References

1. HSE HPSC Study Co-ordination Team: Aoife Colgan FC, Patricia Garvey, Laura et al. (Principal Investigator). SCOPI Steering Group: Derval Igoe DIB, Fiona O'Callaghan, Aoife Colgan FC et al (2020) Preliminary report of the results of the Study to Investigate COVID-19 Infection in People Living in Ireland (SCOPI): a national seroprevalence study
2. Public Health England (2020) Enhanced seroIncidence for COVID-19 antibodies among PHE and NHS Staff (ESCAPE). Study Protocol Version 1.2. England
3. Allen N, Riain UN, Conlon N et al (2021) Prevalence of antibodies to SARS-CoV-2 in Irish hospital healthcare workers. Epidemiol Infect 1–33
4. Walsh EE, Frenck RW Jr, Falsey AR et al (2020) Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. New Eng J Med
5. Vabret N, Britton GJ, Gruber C et al (2020) Immunology of COVID-19: current state of the science. Immunity
6. Patel MM, Thornburg NJ, Stubblefield WB et al (2020) Change in antibodies to SARS-CoV-2 over 60 days among health care personnel in Nashville, Tennessee Jama 324(17):1781–1782
7. Gudbjartsson DF, Norddahl GL, Melsted P et al (2020) Humoral immune response to SARS-CoV-2 in Iceland. N Engl J Med 383(18):1724–1734
8. Muecksch F, Wise H, Batchelor B et al (2020) Longitudinal analysis of clinical serology assay performance and neutralising antibody levels in COVID19 convalescents. MedRxiv
9. Lumley SF, Wei J, O'Donnell D et al (2020) The duration, dynamics and determinants of SARS-CoV-2 antibody responses in individual healthcare workers. MedRxiv
10. Ainsworth M, Andersson M, Auckland K et al (2020) Performance characteristics of five immunoassays for SARS-CoV-2: a head-to-head benchmark comparison. Lancet Infect Dis 20(12):1390–1400