Prevalence of COVID-19 Antibodies in Irish Healthcare Workers (PRECISE 4): November 2021

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(Prevalence of COVID-19 Antibodies in Irish Healthcare Workers)

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Contents

Abbreviations

aRR	adjusted relative risk
ANOVA	Analysis of Variance
anti-spike	anti-S
anti-nucleocapsid	anti-N
BSL3	biosafety level 3
CI	confidence interval
COI	cut-off index
COVID-19	coronavirus disease 2019
EEA	European Economic Area
ELISA	enzyme-linked immunosorbent assay
EU	European Union
HCW	healthcare workers
HPSC	Health Protection Surveillance Centre
HSE	Health Service Executive
IPC	infection prevention and control
IQR	interquartile range
NAb	neutralising antibodies
NTD	N-terminal domain
PPE	personal protective equipment
PRECISE	Prevalence of COVID-19 Antibodies in Irish Healthcare Workers
RBD	receptor binding domain
RR	relative risk
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SD	standard deviation
SJH	St. James's Hospital
UHG	University Hospital Galway
VOC	variants of concern

Summary

Background

Healthcare workers (HCW) constitute a high-risk population for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral transmission. This study was conducted as continuation of a series of multicentre cross-sectional seroprevalence studies entitled PRECISE, for which two hospital sites in areas of Ireland with diverging community incidence and seroprevalence were identified for a multi-site cross-sectional seroprevalence study of anti-SARS-CoV-2 antibodies. This study aimed to assess factors associated with SARS-CoV-2 seropositivity and changes over time, and to assess the durability of antibody responses in a highly vaccinated HCW population in November 2021.

Methods

This seroprevalence study of anti-SARS-CoV-2 antibodies in HCW was conducted in November 2021. HCW were invited to take part and written consent to participate was obtained. HCW were asked to complete a questionnaire on demographics, work-related factors and COVID-19 vaccination status and history, and to undergo serology sampling. Anti-nucleocapsid (N) antibodies and anti-spike (S) antibodies were measured using the Roche Elecsys Anti-SARS-CoV-2 and Roche Elecsys-S Anti-SARS-CoV-2 assays. Paired serology of those HCW participating in the previous study phases in April 2021 and consenting to linkage of data informed an assessment of change in serostatus over time. Seropositivity were classified as (1) unvaccinated and anti-S antibody positive plus anti-N antibody positive or anti-S antibody positive alone or anti-N antibody positive alone, or (2) vaccinated and anti-S antibody plus anti-N antibody positive. Seropositivity was quantified for the total study population and by hospital site. Risk factors for SARS-CoV-2 were assessed for participant characteristics relating to demographics and work-related factors and adjusted relative risks (aRR) and 95% confidence intervals (CI) were calculated in multivariable analysis.

Results

Data from a total of 2,344 HCW in the two hospital sites were analysed, with 75% of the participating HCW working in SJH. Majority (80.5%) were female and median age was 43 years (IQR 33-50). The ethnic groups of Irish (white) (78.9%) and Asian (12.3%) were most commonly reported, and nursing or midwifery staff (39.3%) were the most prevalent staff roles. Nearly all had completed a primary series of vaccination (97.7%), with Comirnaty (formerly Pfizer; 82.3%) and Vaxzevria (formerly AstraZeneca; 16.3%) as the most common vaccine brands received. SARS-CoV-2 seroprevalence for the total study population was 23.4% (n=548), of which 33.6% (n=184) represented undiagnosed infections. All vaccinated HCW had detectable anti-S antibodies. No participating HCW with paired serology demonstrated loss of their anti-S positivity since the previous study phase (April 2021), while 8.8% lost their anti-N positivity. Risk factors for SARS-CoV-2 seropositivity included age 18-29 years (aRR 1.50, 95% CI: 1.19–1.90) compared to age 50-59 years, India as country of birth (aRR 1.35, 95% CI: 1.01–1.73) compared to Ireland, primary/secondary/third level education (aRR 1.35, 95% CI: 1.11–1.66) compared to post-graduate, and being a health care assistant (aRR 2.12, 95% CI: 1.51–2.95) compared to administrative staff.

Conclusion

An increase in SARS-CoV-2 seroprevalence from April to November 2021, reflected the magnitude of the Delta pandemic wave in Ireland. Persistently higher seroprevalence was observed in one hospital site situated in a higher density area, with higher community SARS-CoV-2 incidence. Increased risk for SARS-CoV-2 seropositivity among primarily younger ages, healthcare assistants and those with lower educational level persisted. All vaccinated HCW maintained their anti-S positivity prior to COVID-19 booster vaccination, while anti-N positivity was more dynamic over time.

Main body

Background

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in December 2019 in Wuhan, China, and rapidly spread globally to cause the most significant pandemic (since the 1918 influenza pandemic), with more than 450 million cases reported worldwide within the first two years and more than 100 million cases in the European Union (EU)/European Economic Area (EEA) alone (1). With progression of the coronavirus disease 2019 (COVID-19) pandemic, it became clear that hospital settings constituted an important setting for SARS-CoV-2 viral transmission. Healthcare workers (HCW) and their households are evidently at increased risk of SARS-CoV-2 infection (2-4). Several risk factors have been identified, including but not limited to male gender (5-7), the job roles of health care assistants (HCA) and nurses (6-9), shortage of personal protective equipment (PPE) (3, 4, 6), ethnicity (5-7, 10), and lower educational level (5).

Natural infection with SARS-CoV-2 is generally known to elicit antibodies to both nucleocapsid (N) and spike (S) proteins (11), while most available COVID-19 vaccines (e.g. mRNA or viral vector) target the S protein only (12). As such, the detection of anti-N-antibodies allows to distinguish vaccine-induced seroconversion from antibodies elicited by natural infection (13). Natural infection has been shown to produce humoral and cellular immunity. While previous SARS-CoV-2 infection has shown some protection against re-infection, protection may decline over extended time (14-16). COVID-19 vaccines have demonstrated protection against severe disease (17-19) and reduced incidence of symptomatic infection (19), although considerable waning of immunity six months after receiving a second vaccine dose (primary series) and reduction in anti-SARS-CoV-2 antibodies have been shown over time (20, 21). The SARS-CoV-2 S protein mediates viral entry into the host cell via the host ACE2 receptor and is a critical target for neutralising antibodies (NAb). NAb play a key role in primary prevention of infection and viral clearance (22). Crucial targets within the S protein include the receptor binding domain (RBD) and N-terminal domain (NTD) on the S1 subunit (22). Accurate determination of virus neutralisation is challenging owing to the requirement for biosafety level 3 (BSL3) facilities utilising live SARS-CoV-2 viral models and as a result, surrogate lentiviral vectors pseudotyped with the SARS-CoV-2 spike protein are often adopted (22, 23).

At the time of the study conduct, five variants of concern (VOC) had been identified in Ireland including B.1.1.7 (Alpha), B.1.351 (Beta), G.1 (Gamma), B.1.617.2 (Delta) and B.1.1.529 (Omicron) (24). The fourth pandemic wave in Ireland between week 26 (27th June to 3rd July) and week 50 (12th to 18th December 2021) was dominated by the circulation of the Delta VOC, while the fifth pandemic wave commenced in week 51, 2021, when the Omicron VOC became

the dominant circulating variant (25). With pandemic progression and emergence of VOC, more individuals developed hybrid immunity (i.e. immunity from SARS-CoV-2 infection and vaccination in combination). Evidence shows that protection provided by hybrid immunity against severe disease remains high up to 12 months after primary vaccination series, while protection against re-infection and symptomatic disease substantially decreases by six months (26, 27). Increasing evidence on waning immunity raised concerns relating to the durability of antibody responses and duration of vaccine protection in 2021, which triggered the implementation of COVID-19 booster vaccination programmes. In Ireland, the COVID-19 booster vaccination was extended to include HCW on 2nd November 2021 (28).

Understanding the transmission and immunity dynamics of SARS-CoV-2 in HCW and by association with hospital environments in Ireland remained of importance throughout the pandemic, including adding to the evidence on risk factors for SARS-CoV-2 infection and duration of vaccine induced immunity among HCW. Two hospital sites, St. James's Hospital (SJH) and University Hospital Galway (UHG), with diverging community incidence and seroprevalence were identified for a multicentre cross-sectional seroprevalence study entitled Prevalence of COVID-19 Antibodies in Irish Healthcare Workers (PRECISE) with the first study phase in 2020. The PRECISE 4 study was conducted in November 2021 as a continuation of a series of studies, that included PRECISE 1 in October 2020 and PRECISE 2 in April 2021 following COVID-19 vaccination roll-out. The PRECISE 4 study was a cross-sectional seroprevalence study of SARS-CoV-2 specific antibodies in HCW prior to receipt of the COVID-19 booster vaccination. The aim of this study was to assess factors associated with SARS-CoV-2 seropositivity and changes over time, as well as to assess the durability of antibody responses in a highly vaccinated HCW population. This report complements the already published peer-reviewed paper on the PRECISE 4 study (23).

Methodology

Study design and participants

This was a multi-site cross-sectional seroprevalence study of anti-SARS-CoV-2 antibodies in HCW in two hospital sites in Ireland conducted between the 10th and 23rd November 2021. HCW who participated in the previous serosurvey (PRECISE 2) were invited to take part in the PRECISE 4 study through internal hospital communication, emails, and text messages. Similar to previous PRECISE study phases (9, 29), participants were asked to complete a questionnaire developed by the study team and undergo serology sampling. Enrolment was open to HCW prior to receipt of booster COVID-19 vaccination, and to those partially vaccinated or unvaccinated.

Written consent (including consent to link with PRECISE 1 and 2 results) was obtained following review of participant information leaflets (Appendix A). All participating HCW were asked to complete a written questionnaire (Appendix B; shorter version than previous study phases) collecting demographics, work-related factors, and COVID-19 previous infection and vaccination history. Due to time constraints resulting from the rapid roll-out of the COVID-19 booster vaccination programme in HCW in November 2021 (28), the questionnaire was not pilot tested or distributed in languages other than English.

HCW were categorised according to the self-reported role undertaken at the hospital at the time of completing the written questionnaire. Allied healthcare was defined as those health or related services pertaining to the identification, evaluation and prevention of disease e.g.

dietary and nutrition services, rehabilitation and health systems management. Staff roles such as catering, cleaning, maintenance, and security were re-categorised as general support staff, while staff working within ambulance services, education, research and IT or technical support were defined as other staff roles.

Study sites

SJH is a tertiary hospital in the south inner city of Dublin, the capital of Ireland, with approximately 4,700 employees and over 1,000 beds. Between March and May 2020 (first wave of the COVID-19 pandemic in Ireland (30)), 9.6% of the staff of SJH tested positive for SARS-CoV-2 infection via polymerase chain reaction (PCR), and by the start of October 2020 (start of the second wave of the pandemic in Ireland (30)), 10.2% of staff had tested positive by PCR(9). UHG is a comparable tertiary hospital in Galway in the West of Ireland, with approximately 4,400 employees and over 500 beds. During the time-period from March to May 2020, 1.8% of the staff of UHG had a PCR-confirmed SARS-CoV-2 infection at some point, and this remained at 1.8% until the start of October 2020. Both hospitals received patients with COVID-19 infection throughout the pandemic, and breakdown by ward and specialty is similar. The community incidence of COVID-19 infection in County Galway was significantly lower than in County Dublin during this time period, which covered the first wave of the pandemic in Ireland and the start of the second wave (30). A community seroprevalence study observed significantly lower seroprevalence County Sligo in the West of Ireland (0.6%) as compared to the County Dublin (3.1%) in June 2020 (31).

Serology sampling and laboratory methods

Serology sampling was carried out in the period from 10th - 23rd November 2021 in SJH and 10th – 12th November 2021 in UHG. Blood samples were analysed by laboratories in each hospital site using the Roche Elecsys Anti-SARS-CoV-2 assay for detection of anti-nucleocapsid (N) antibodies (as a measure of previous infection) and the Roche Elecsys-S Anti-SARS-CoV-2 assay for detection of anti-Spike (S) antibodies (as a measure of vaccine response). As per the manufacturer guidelines on the titre level thresholds, a cut-off of ≥1.0 cut-off index (COI) in the Roche Elecsys Anti-SARS-CoV-2 assay and ≥0.8 U/mL in the Roche Elecsys-S Anti-SARS-CoV-2 assay was considered positive (32, 33).

Participants with detectable anti-N antibodies and/or anti-S antibodies and no reported COVID-19 vaccination history were presumed to have had a previous SARS-CoV-2 infection (natural infection). In participants with a COVID-19 vaccination history, detectable anti-S antibodies was assumed anti-S positive in response to vaccination and detectable anti-N antibodies was assumed anti-N positive in response to previous SARS-CoV-2 infection. Paired serology results from PRECISE 2 in April 2021, for those who consented to linkage of data, informed an analysis of change in serostatus over time.

The presence of neutralising antibodies capable of blocking interaction between S protein-RBD and ACE2 was investigated in a subset of samples representative of the larger cohort, via an in vitro ACE2 binding enzyme-linked immunosorbent assay (ELISA). Methods of sample selection are detailed in Supplementary material A, section ii), to the published PRECISE 4 paper (23). This surrogate neutralisation assay demonstrated the extent to which biotinylated ACE2 interaction with S-RBD is inhibited by antibodies in participant sera and has demonstrated close correlation with spike-based pseudovirus infection assays for the assessment of ACE2-RBD binding neutralisation. Full details of this assay including

comparator results to other assays are published elsewhere (34), see also Supplementary material A to the published PRECISE 4 paper for further details (23).

Classification of seropositivity and undiagnosed infections

A study participant was classified as seropositive if fulfilling one of the following: (1) unvaccinated and anti-S antibody positive plus anti-N antibody positive or anti-S antibody positive alone or anti-N antibody positive alone, or (2) vaccinated and anti-S antibody plus anti-N antibody positive. Undiagnosed infections were defined as those seropositive with no self-reported previous laboratory-confirmed (by PCR) COVID-19 infection.

Classification of vaccination status and breakthrough infections

Participants received their COVID-19 vaccine as part of a two-dose vaccination primary series of either the Comirnaty (formerly Pfizer) or Spikevax (formerly Moderna) mRNA vaccines, the Vaxzevria (formerly AstraZeneca) viral vector vaccine or a one-dose vaccination primary series of the Janssen viral vector vaccine. Participants with a completed primary series of vaccination were defined as ≥14 days after receipt of the second COVID-19 vaccine dose of a two-dose vaccination course or first COVID-19 vaccine dose if receiving the Janssen COVID-19 vaccine. Subjects with partially completed primary series of vaccination were defined as ≥14 days after receipt of the first COVID-19 vaccine dose of a two-dose vaccination primary series. Owing to some uncertainty relating to participant self-reported COVID-19 vaccine dates, dates were not considered for participants where the time interval between first and second vaccine date were either negative, zero or <17 days as per the National Immunisation Advisory Committee (NIAC) guidelines of a valid second vaccine dose, at the time the study was conducted (35). Vaccination status presented as partially and fully completed primary series of vaccination was further classified according to participant self-reported receipt of all required vaccine doses.

Breakthrough infections were defined as participant self-reported PCR confirmed SARS-CoV-2 infection ≥14 days after completing a primary series of vaccination, or self-reported PCR confirmed SARS-CoV-2 infection ≥14 days after receiving a first vaccine primary series dose or <14 days after receiving a second vaccine dose of a two-dose regime.

Statistical analysis

Frequencies and percentages were calculated for participant characteristics for the total study population and by hospital. Comparisons between hospital sites were made using the Chi-square test. The prevalence of SARS-CoV-2 seropositivity was calculated according to participant characteristics for the total study population and by hospital. Univariable and multivariable logistic regression (log-binomial regression) provided crude and adjusted relative risks (RR) and their 95% confidence intervals (CI) in order to assess the association between relevant factors and SARS-CoV-2 seropositivity. Following univariable analysis, variables with a p-value <0.05 were considered in the multivariable analysis. A sensitivity analysis showed that the removal of missing data did not substantially change the absolute magnitude of estimates, and a complete-case analysis was deemed appropriate for consistency in comparison of the regression models. We followed a stepwise forward selection of variables using analysis of variance (ANOVA) to determine if the addition of a variable significantly improved the model fit. For those participants taking part in PRECISE 2 and PRECISE 4 (paired sera), comparison of the median antibody titres between study phases was undertaken

using the Mann-Whitney U test for variables with two levels and the Kruskal-Wallis test with Dunn's multiple correction test for multi-level variables. Participant characteristics in the ACE2RBD binding sub-group were compared to the larger cohort using the Chi-square test. The Mann-Whitney U test and Kruskal-Wallis test with Dunn's multiple test correction was used to compare the ACE2-RBD neutralisation observed by participant characteristics. All analyses were carried out using R (version 4.1.3, R Core Team 2021).

Ethical approval and funding

Ethical approval was obtained from the National Research Ethics Committee (NREC) with application number 20-NRECCOV-101-AMEND-2. This work was supported financially by the Health Service Executive (HSE) COVID-19 budget. The funders had no role in the study design or in the data analysis and interpretation.

Results

Study participation

All HCW participating in PRECISE 2 were invited to take part in PRECISE 4. Following recruitment and serology sampling, a total of 2,415 HCW were included in the two hospital sites. Of those, 71 participants were excluded from the analysis due to already having received a COVID-19 booster vaccination, which resulted in a total study population of 2,344 HCW. By hospital site, 1,778 (75.9%) HCW from SJH and 566 (24.1%) HCW from UHG participated. Of those participating in PRECISE 4, 94% (n=2,208) HCW had also taken part in PRECISE 2.

Demographics

Participant characteristics are presented in **Table 1**. Sex and level of education were similar between the hospital sites. The majority (80.5%) were female, and the median age was 43 years (IQR 33-50). Age groups, ethnicity, country of birth, and staff role differed between the hospital sites. By ethnicity, 78.9% were Irish (white) (77.3% in SJH; 84.1% in UHG) followed by 12.3% Asian (14.8% in SJH; 4.4% in UHG) and 7.1% other white background (6.5% in SJH; 9.0% in UHG). Just over one third (39.3%) were nursing or midwifery staff, while 22.1% were allied healthcare staff and 15.4% administration staff. Medical/dental, general support, HCA, and other staff roles were reported by less than ten percent, with some noticeable differences between hospital sites (**Table 1**).

Table 1 Participant characteristic by hospital and total study population, PRECISE 4, Ireland, November 2021

Participant characteristics		St James's Hospital (N=1,778)		University Hospital Galway (N=566)		P-value*	Total (N=2,344)	
		n	%	n	%		N	%
Age (years)	Mean (SD)	41.8 (11.2)		42.2 (10.3)		-	41.9 (11.0)	
	Median (IQR)	42 (32 – 50)		43 (34 – 50)			43 (33 – 50)	
Age groups (years)	18-29	311	17.5	74	13.1	**0.030**	385	16.4
	30-39	433	24.4	152	26.9		585	25.0
	40-49	533	30.0	192	33.9		725	30.9
	≥ 50	501	28.2	148	26.1		649	27.7
Sex	Female	1,414	79.5	472	83.4	0.050	1,886	80.5
	Male	364	20.5	94	16.6		458	19.5
Ethnicity	Irish (white)	1,374	77.3	476	84.1	**<0.001**	1,850	78.9
	Any other white background	116	6.5	51	9.0		167	7.1
	Asian background	263	14.8	25	4.4		288	12.3
	African and other black background	23	1.3	8	1.4		31	1.3
	Unknown	2	0.1	6	1.1		8	0.3
Country of birth	Ireland	1,297	73.0	439	77.5	**<0.001**	1,736	74.0
	Philippines	127	7.1	4	0.7		131	5.6
	India	120	6.7	9	1.6		129	5.5
	United Kingdom	71	4.0	36	6.4		107	4.6
	Other	163	9.2	78	13.8		241	10.3
Education	Primary	3	0.2	0	0.0	0.299	3	0.1
	Secondary	192	10.8	53	9.4		245	10.5
	Third level†	973	54.7	341	60.2		1,314	56.1
	Post-graduate	471	26.5	146	25.8		617	26.3
	Missing	139	7.8	26	4.6		165	7.0
Role	Administration	260	14.6	102	18.0	**0.019**	362	15.4
	Medical/dental	154	8.6	44	7.8		198	8.5
	Nursing/midwifery	709	39.9	213	37.6		922	39.3
	Allied health	396	22.3	121	21.4		517	22.1
	General support	110	6.2	25	4.4		135	5.8
	Healthcare assistant	79	4.4	25	4.4		104	4.4
	Other	42	2.4	27	4.8		69	2.9
	Missing	28	1.6	9	1.6		37	1.6

Values are n and % unless otherwise indicated. IQR = interquartile range, SD = standard deviation.

* Calculated using the chi-square test

† Third level education excludes Post-graduate (NFQ Levels Level 9-10)

COVID-19 previous infection and vaccination status

On combined hospital data, 20.3% (n=475) self-reported a previous laboratory-confirmed (by PCR) COVID-19 infection (**Table 2**). Of those participants, 81.7% (n=388) self-reported a previous infection before receiving any COVID-19 vaccine dose (natural infections), while 3.6% (n=17) represented breakthrough infections following one vaccine dose of a two-dose primary vaccination series , and 14.7% (n=70) represented breakthrough infections following completion of a primary vaccination series . The median time between completed primary series of vaccination and participant self-reported previous laboratory-confirmed (by PCR) COVID-19 infection was 202 (IQR 155-244) days. Approximately 66% of breakthrough infections following completion of primary vaccination series occurred among participants in SJH (**Table 3**). Those with breakthrough infections were similar in demographics to the total study population, with a median age of 43.5 (IQR 32.5-49) years, 80.0% female and the majority originating from Ireland.

In terms of COVID-19 vaccination status of the participating HCW, 98.7% (n=2,313) were vaccinated (with any dose), 1.2% (n=30) were unvaccinated, and 0.1% (n=1) had unknown vaccination status. The majority (81.1%) had received the Comirnaty vaccine, followed by the Vaxzevria vaccine (16.1%), with proportional differences between hospital sites. The breakdown of vaccine types is also shown in **Table 2**. The median time between completed primary series of vaccination and serology sampling was 298 (IQR 281-300) days.

Table 2 COVID-19 related characteristics by hospital and total study population, PRECISE 4, Ireland, November 2021

Participant characteristics		**St James's Hospital (N=1,778)**		**University Hospital Galway (N=566)**		**P-value***	**Total (N=2,344)**	
		n	**%**	**n**	**%**		**N**	**%**
Previous positive COVID-19 PCR test	Yes	353	19.9	122	21.6	0.414	475	20.3
	No	1,425	80.1	444	78.4		1,869	79.7
Seropositive †	Yes (seropositive)	429	24.1	119	21.0	0.266	548	23.4
	No (seronegative)	1,348	75.8	447	79.0		1,795	76.6
	Unknown	1	0.1	0	0.0		1	0.0
Undiagnosed infections ‡	Yes (undiagnosed)	163	9.1	21	3.7	**<0.001**	184	7.8
	No (other)	1,614	90.8	545	96.3		2,159	92.1
	Unknown	1	0.1	0	0.0		1	0.1
Vaccination status	Vaccinated (any dose)	1,759	98.9	554	97.9	0.106	2,313	98.7
	Unvaccinated	18	1.0	12	2.1		30	1.2
	Unknown	1	0.1	0	0.0		1	0.1
Vaccine brand	Comirnaty	1,421	79.9	481	85.0	**<0.001**	1,902	81.1
	Vaxzevria	322	18.1	55	9.7		377	16.1
	Other	15	0.8	17	3.0		32	1.4
	None (unvaccinated)	18	1.0	12	2.1		30	1.3
	Unknown	2	0.1	1	0.2		3	0.1
Vaccine type	mRNA (Comirnaty, Spikevax)	1,434	80.7	495	87.5	**<0.001**	1,929	82.3

Participant characteristics		St James's Hospital (N=1,778)		University Hospital Galway (N=566)		P-value*	Total (N=2,344)	
		n	%	n	%		N	%
	Viral vector (Vaxzevria, Janssen)	323	18.2	57	10.1		380	16.2
	Heterologous doses ¶	1	0.1	1	0.2		2	0.1
	None (unvaccinated)	18	1.0	12	2.1		30	1.3
	Unknown	2	0.1	1	0.2		3	0.1

COVID-19 = coronavirus disease-19, PCR = polymerase chain reaction.
* Calculated using the chi-square test. **Bold** values indicate significant P-values.
† A participant was classified as seropositive if (1) unvaccinated and anti-spike (S) antibody plus anti-nucleocapsid (N) antibody positive or anti-S antibody positive alone or anti-N antibody positive alone, or (2) vaccinated and anti-S antibody plus anti-N antibody positive.
‡ Undiagnosed infections were defined as those seropositive with no self-reported previous laboratory-confirmed (by PCR) COVID-19 infection.
¶ Heterologous doses refer to participants who received different COVID-19 vaccine brands/types for the first and second dose.

Table 3 Characteristics of participants with breakthrough infections following completion of a primary series of vaccination, PRECISE 4, Ireland, November 2021 (N=70) *

Participant characteristics		St James's Hospital (N=46)		University Hospital Galway (N=24)		P-value†	Total (N=70)	
		n	%	n	%		N	%
Hospital	St James's Hospital	-	-	-	-	-	46	65.7
	University Hospital Galway	-	-	-	-		24	34.3
Age (years)	Mean (SD)	41.2 (10.9)		43.8 (8.2)		-	42.1 (10.0)	
	Median (IQR)	43 (30-49)		44 (38-47)			43.5 (32.5-49)	
Age groups (years)	18-29	8	17.4	1	4.2	0.615	9	12.9
	30-39	11	23.9	6	25.0		17	24.3
	40-49	16	34.8	11	45.8		27	38.6
	50-59	9	19.6	5	20.8		14	20.0
	≥ 60	2	4.3	1	4.2		3	4.3
Sex	Female	38	82.6	18	75.0	0.660	56	80.0
	Male	8	17.4	6	25.0		14	20.0
Ethnicity	Irish (white)	35	76.1	19	76.0	0.191	54	77.1
	Any other white background	1	2.2	3	16.0		4	5.7
	Asian background	9	19.6	2	8.0		11	15.7
	African and other black background	1	2.2	0	0.0		1	1.4
	Unknown	0	0.0	0	0.0		0	0.0
Country of birth	Ireland	35	76.1	17	70.8	0.135	52	74.3
	Philippines	6	13.0	0	0.0		6	8.6
	India	3	6.5	3	12.5		6	8.6
	United Kingdom	1	2.2	1	4.2		2	2.9
	Other	1	2.2	3	12.5		4	5.7
Education	Primary	0	0.0	0	0.0	0.085	0	0.0

Participant characteristics		St James's Hospital (N=46)		University Hospital Galway (N=24)		P-value†	Total (N=70)	
		n	%	n	%		N	%
	Secondary	8	17.4	0	0.0		8	11.4
	Third level‡	23	50.0	13	54.2		36	51.4
	Post-graduate	12	26.1	9	37.5		21	30.0
	Missing	3	6.5	2	8.3		5	7.1
Role	Administration	10	21.7	3	12.6	0.361	13	18.6
	Medical/dental	3	6.5	1	4.2		4	5.7
	Nursing/midwifery	18	39.1	8	33.3		26	37.1
	Allied health	6	13.0	8	33.3		14	20.0
	General support	2	4.4	2	8.3		4	5.7
	Healthcare assistant	2	4.4	2	8.3		4	5.7
	Other	3	6.5	0	0.0		3	4.3
	Missing	2	4.4	0	0.0		2	2.9
Vaccine brand	Comirnaty	46	100.0	22	91.7	0.139	68	97.1
	Vaxzevria	0	0.0	1	4.2		1	1.4
	Other	0	0.0	1	4.2		1	1.4
Vaccine type	mRNA (Comirnaty, Spikevax)	48	100.0	23	95.8	0.739	69	98.6
	Viral vector (Vaxzevria, Janssen)	0	0.0	1	4.2		1	1.4
	Heterologous doses	0	0.0	0	0.0		0	0.0

IQR = interquartile range, SD = standard deviation

* Defined as PCR confirmed SARS-CoV-2 infection ≥14 days after receiving 2nd COVID-19 vaccine dose (≥14 days after completion of primary series of vaccination)

† Calculated using the chi-square test

‡ Third level education excludes Post-graduate (NFQ Levels Level 9-10)

SARS-CoV-2 seroprevalence

SARS-CoV-2 seroprevalence for the total study population and by hospital is presented in **Table 4a-c**. The prevalence of SARS-CoV-2 seropositive participants combined for both hospital sites was 23.4% (n=548) (**Table 4a**). Of those, 33.6% (n=184) did not report having had a previous laboratory-confirmed COVID-19 infection, representing nearly 8% of the total study population with an undiagnosed infection, which was significantly higher in SJH than UHG (**Table 2**). The SARS-CoV-2 seroprevalence was 24.1% in SJH and 21.0% in UHG. The combined data for both hospitals showed the highest prevalence of SARS-CoV-2 seropositivity in the age group 18-29 years (30.6%, 95% CI: 26.2-35.4) (**Table 4a**). Similarly, the participating HCW aged 18-29 years in SJH had a prevalence of SARS CoV-2 seropositivity at 32.2% (95% CI: 27.2 – 37.6). The differences in prevalence between age groups in UHG was less pronounced. By ethnic group, the highest seroprevalence was found amongst those of African and other black background (32.3%, 95% CI: 18.1-50.6), followed by Irish white (21.8%, 95% CI: 20.0-23.7). This was explained by the prevalence of seropositivity among African and other black background in SJH (43.5%, 95% CI: 24.9 - 64.1), as no HCW of this ethnic group (0/8) was found to be seropositive in UHG. By country of birth,

the highest seroprevalence was found in participating HCW born in India (32.6%, 95% CI: 25.0-41.1) and the Philippines (29.8%, 95% CI: 22.5-38.2), although it should be noted that fewer participants were born in countries other than Ireland. The seroprevalence decreased with increasing level of education ranging from 66.7% (95% CI: 9.5-97.4) among those with primary level of education to 16.9% (95% CI: 14.1-20.0) among those with post-graduate level of education. Similar tendencies were shown for the two hospital sites, except that no participants in UHG reported primary education level only (**Tables 4b-c**). With regards to staff roles, the highest prevalence of SARS-CoV-2 seropositivity was found among HCA, with 42.3% (95% CI: 33.2 - 52.0) seropositive. For individual staff roles in SJH, the seroprevalence was highest in HCA (43.0%) followed by nursing/midwifery (27.2%), general support staff (26.6%), other staff roles (26.2%), administrative staff (22.3%), allied health staff (18.4%) and finally medical/dental staff (16.2%) (**Table 4b**). In UHG, the seroprevalence was also highest in HCA (40.0%) but followed by medical/dental staff (29.5%), general support staff (28.0%), nursing/midwifery (24.9%), other staff roles (22.2%), allied health staff (13.2%) and administrative staff (11.8%) (**Table 4c**).

Table 4a Prevalence of SARS-CoV-2 seropositivity by participant characteristics, both hospitals, PRECISE 4, Ireland, November 2021 (N=2,343) *

Participant characteristics		Total	SARS-CoV-2 seropositivity		
		N	n	% (95% CI)	P-value†
Overall		2,343	548	23.4 (21.7 - 25.1)	-
Hospital	St. James's Hospital	1777‡	429	24.1 (22.2 - 26.2)	0.419
	University Hospital Galway	566	119	21.0 (17.9 - 24.6)	
Age groups (years)	18-29	385	118	30.6 (26.2 - 35.4)	**0.002**
	30-39	584	122	20.9 (17.8 -24.4)	
	40-49	725	160	22.1 (19.2 - 25.2)	
	≥ 50	649	148	22.8 (19.7 – 26.2)	
Sex	Female	1,885	436	23.1 (21.3 - 25.1)	0.590
	Male	458	112	24.5 (20.7 - 28.6)	
Ethnicity	Irish (white)	1,850	403	21.8 (20.0 - 23.7)	**0.012**
	Any other white background	167	50	29.9 (23.5 - 37.3)	
	African and other black background	31	10	32.3 (18.1 - 50.6)	
	Asian background	287	83	28.9 (24.0 - 34.4)	
	Unknown	8	2	25.0 (5.7 - 65.0)	
Country of birth	Ireland	1,736	379	21.8 (19.9 - 80.1)	**0.011**
	Philippines	131	39	29.8 (22.5 - 38.2)	
	India	129	42	32.6 (25.0 - 41.1)	
	United Kingdom	107	21	19.6 (13.1 - 28.3)	
	Poland	33	9	27.3 (14.7 - 45.0)	
	USA	14	2	14.3 (3.4 - 44.1)	
	Other	193	56	29.0 (23.0 - 35.8)	
Education	Primary	3	2	66.7 (9.5 - 97.4)	**<0.001**
	Secondary	245	78	31.8 (26.3 - 37.9)	
	Third level¶	1,314	320	24.4 (22.1 - 26.8)	
	Post-graduate	617	104	16.9 (14.1 - 20.0)	
	Missing	164	44	26.8 (20.6 - 34.1)	
Role	Administration	362	70	19.3 (15.6 - 23.7)	**<0.001**
	Medical/dental	198	38	19.2 (14.3 - 25.3)	
	Nursing/midwifery	922	246	26.7 (23.9 - 29.6)	
	Allied health	517	89	17.2 (14.2 - 20.7)	
	General support	134	36	26.9 (20.0 - 35.0)	
	Healthcare assistant	104	44	42.3 (33.2 - 52.0)	
	Other	69	17	24.6 (15.8 - 36.2)	
	Missing	37	8	21.6 (11.1 - 37.9)	

CI = confidence interval, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

* A participant was classified as seropositive if (1) unvaccinated and anti-spike (S) antibody plus anti-nucleocapsid (N) antibody positive or anti-S antibody positive alone or anti-N antibody positive alone, or (2) vaccinated and anti-S antibody plus anti-N antibody positive.

† Calculated using the chi-squared test. **Bold** values indicate significant P-values.

‡ Seropositivity unknown for one subject.

¶ Third level education excludes Post-graduate (NFQ Levels Level 9-10)

Table 4b Prevalence of SARS-CoV-2 seropositivity by participant characteristics, St. James's Hospital, PRECISE 4, Ireland, November 2021*

Participant characteristics		Total	SARS-CoV-2 seropositivity		
		N	n	% (95% CI)	P-value†
Overall		1777 ‡	429	24.1 (22.2 - 26.2)	-
Age groups (years)	18-29	311	100	32.2 (27.2 - 37.6)	**0.003**
	30-39	432	92	21.3 (17.7 - 25.4)	
	40-49	533	122	22.9 (19.5 - 26.7)	
	≥ 50	501	115	23.0 (19.5 - 26.8)	
Sex	Female	1,413	339	24.0 (21.8 - 26.3)	0.824
	Male	364	90	24.7 (20.6 - 29.4)	
Ethnicity	Irish (white)	1,374	304	22.1 (20.0 - 24.4)	**0.003**
	Any other white background	116	36	31.0 (23.3 - 40.1)	
	Asian background	262	78	29.8 (24.5 - 35.6)	
	African and other black background	23	10	43.5 (24.9 - 64.1)	
	Unknown	2	1	50.0 (1.9 - 98.1)	
Country of birth	Ireland	1,297	287	22.1 (19.9 - 24.5)	**0.030**
	Philippines	127	39	30.7 (23.3 - 39.3)	
	India	120	37	30.8 (23.2 - 39.7)	
	United Kingdom	71	16	22.5 (14.2 - 33.8)	
	Poland	10	2	20.0 (4.6 - 56.2)	
	USA	5	1	20.0 (2.1 - 74.4)	
	Other	147	47	32.0 (24.9 - 40.0)	
Education	Primary	3	2	66.7 (9.55 - 97.4)	**<0.001**
	Secondary	192	63	32.8 (26.5 - 39.8)	
	Third level¶	973	252	25.9 (23.2 - 28.7)	
	Post-graduate	471	76	16.1 (13.1 - 19.7)	
	Missing	138	36	26.1 (19.4 - 34.1)	
Role	Administration	260	58	22.3 (17.6 - 27.8)	**<0.001**
	Medical/dental	154	25	16.2 (11.2 - 23.0)	
	Nursing/midwifery	709	193	27.2 (24.1 - 30.6)	
	Allied health	396	73	18.4 (14.9 - 22.6)	
	General support	109	29	26.6 (19.1 - 35.7)	
	Healthcare assistant	79	34	43.0 (32.5 - 54.2)	
	Other	42	11	26.2 (15.0 - 41.6)	
	Missing	28	6	21.4 (9.8 - 40.6)	

CI = confidence interval, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

* A participant was classified as seropositive if (1) Unvaccinated **and** anti-spike (S) antibody plus anti-nucleocapsid (N) antibody positive **or** anti-S antibody positive alone **or** anti-N antibody positive alone, or (2) Vaccinated **and** anti-S antibody plus anti-N antibody positive.

† Calculated using the chi-squared test. **Bold** values indicate significant P-values.

† Seropositivity unknown for one subject.

¶ Third level education excludes Post-graduate (NFQ Levels Level 9-10)

Table 4c Prevalence of SARS-CoV-2 seropositivity by participant characteristics, University Hospital Galway, PRECISE 4, Ireland, November 2021*

Participant characteristics		Total	SARS-CoV-2 seropositivity		
		N	n	% (95% CI)	P-value†
Overall		566	119	21.0 (17.9 - 24.6)	-
Age groups (years)	18-29	74	18	24.3 (15.8 - 35.5)	0.812
	30-39	152	30	19.7 (14.1 - 26.9)	
	40-49	192	38	19.8 (14.7 - 26.1)	
	≥ 50	148	33	22.3 (16.3 - 29.8)	
Sex	Female	472	97	20.6 (17.1 - 24.4)	0.630
	Male	94	22	23.4 (15.9 - 33.1)	
Ethnicity	Irish (white)	476	99	20.8 (17.4 - 24.7)	0.478
	Any other white background	51	14	27.5 (16.9 - 41.4)	
	African and other black background	8	0	-	
	Asian background	25	5	20.0 (8.4 - 40.5)	
	Unknown	6	1	16.7 (1.8 - 67.9)	
Country of birth	Ireland	439	92	21.0 (17.4 - 25.0)	0.106
	Philippines	4	0	-	
	India	9	5	55.6 (23.6 - 83.5)	
	United Kingdom	36	5	13.9 (5.82 - 29.6)	
	Poland	23	7	30.4 (15.0 - 52.1)	
	USA	9	1	11.1 (1.3 -53.3)	
	Other	46	9	19.6 (10.4 - 33.7)	
Education	Primary	0	0	-	0.334
	Secondary	53	15	28.3 (17.7 - 41.9)	
	Third level‡	341	68	19.9 (16.0 - 24.5)	
	Post-graduate	146	28	19.2 (13.6 - 26.4)	
	Missing	26	8	30.8 (15.9 - 51.0)	
Role	Administration	102	12	11.8 (6.78 - 19.7)	**0.003**
	Medical/dental	44	13	29.5 (17.9 - 44.7)	
	Nursing/midwifery	213	53	24.9 (19.5 - 31.2)	
	Allied health	121	16	13.2 (8.24 - 20.6)	
	General support	25	7	28.0 (13.7 - 48.7)	
	Healthcare assistant	25	10	40.0 (22.7 - 60.2)	
	Other	27	6	22.2 (10.2 - 41.9)	
	Missing	9	2	22.2 (5.1 - 60.3)	

CI = confidence interval, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

* A participant was classified as seropositive if (1) Unvaccinated **and** anti-spike (S) antibody plus anti-nucleocapsid (N) antibody positive **or** anti-S antibody positive alone **or** anti-N antibody positive alone, or (2) Vaccinated **and** anti-S antibody plus anti-N antibody positive.

† Calculated using the chi-squared test. **Bold** values indicate significant P-values.

‡ Third level education excludes Post-graduate (NFQ Levels Level 9-10)

Factors associated with SARS-CoV-2 seropositivity

Table 5a presents the results of the univariable and multivariable analysis on the association between risk factors and SARS-CoV-2 seropositivity in the total study population. Hospital site-specific results are shown in **Table 5b-c**. Following univariable analysis, age, ethnicity, country of birth, education, staff role, vaccination status, vaccine brand and type were considered in the multivariable analysis. Ethnicity, vaccination status, vaccine brand and type were excluded from the analysis due to small numbers and resulting multicollinearity in the multivariable analysis. Following a sensitivity analysis, the multivariable analysis was carried out on 2,144 complete cases, with a total of 199 cases dropped due to missing values.

The adjusted RR (aRR) remained significant for the following participant characteristics: age 18-29 years (aRR 1.50, 95% CI: 1.19–1.90) compared to age 50-59, India as country of birth (aRR 1.35, 95% CI: 1.01–1.73) compared to Ireland, primary/secondary/third level education (aRR 1.35, 95% CI: 1.11–1.66) compared to post-graduate, and being a HCA (aRR 2.12, 95% CI: 1.51–2.95) compared to administrative staff (**Table 5a**). The multivariable analysis by hospital site showed that the aRR of SARS-CoV-2 seropositivity in SJH remained significant for age 18-29 years, primary/secondary/third level education and being a HCA (**Table 5b**). In UHG, the aRR remained significant for a number of staff roles. Specifically, compared to administrative roles, the aRR for SARS-CoV-2 seropositivity was higher for medical/dental staff, nurses/midwifes, general support staff and HCA (**Table 5c**).

Table 5a Association between risk factors and SARS-CoV-2 seropositivity, total study population, PRECISE 4, Ireland, November 2021 (N=2,343)

Participant characteristics		n	Unadjusted relative risk (95% CI)	P-value	Adjusted relative risk (95% CI) (complete case analysis, N=2,144)	P-value
Hospital	University Hospital Galway	566	Ref.	**-**		
	St James's Hospital	1777 *	1.15 (0.96 - 1.38)	0.130		
Age groups (years)	18-29	385	1.34 (1.09 - 1.65)	**<0.001**	1.52 (1.21 - 1.90)	**<0.001**
	30-39	584	0.92 (0.74 - 1.12)	0.420	1.01 (0.81 - 1.27)	0.906
	40-49	725	0.97 (0.79 - 1.18)	0.740	1.05 (0.85 - 1.29)	0.679
	≥ 50	649	Ref.	-	Ref.	-
Sex	Female	1,885	Ref.	-		
	Male	458	1.06 (0.88 - 1.26)	0.550		
Ethnicity ‡	Irish (white)	1,850	Ref.	-		
	Any other white background	167	1.25 (0.93 - 1.64)	0.120		
	Asian background	287	1.37 (1.06 - 1.74)	**0.010**		
	African and other black background	31	1.48 (0.81 - 2.30)	0.140		
	Unknown	8	1.15 (0.21 - 2.73)	0.820		
Country of birth	Ireland	1,736	Ref.	-	Ref.	-
	Philippines	131	1.36 (1.01 - 1.77)	**0.030**	1.16 (0.85 - 1.52)	0.324
	India	129	1.49 (1.12 - 1.91)	**<0.001**	1.35 (1.01 - 1.73)	**0.036**
	United Kingdom	107	0.90 (0.58 - 1.29)	0.600	0.93 (0.59 - 1.35)	0.712
	Other	240	1.28 (1.01 - 1.58)	**0.030**	1.12 (0.87 - 1.42)	0.351
Education (N=2,179)	Primary/secondary/third level¶	1,562	1.52 (1.26 - 1.86)	**<0.001**	1.35 (1.11 - 1.66)	**0.004**
	Post-graduate	617	Ref.	-	Ref.	-
Role (N=2,306)	Administration	362	Ref.	-	Ref.	-
	Medical/dental	198	0.99 (0.69 - 1.40)	0.970	0.96 (0.66 - 1.39)	0.350
	Nursing/midwifery	922	1.38 (1.10 - 1.76)	**0.010**	1.28 (0.99 - 1.69)	0.060
	Allied health	517	0.89 (0.67 - 1.18)	0.420	0.87 (0.64 - 1.18)	0.350
	General support	134	1.39 (0.97 - 1.95)	0.070	1.28 (0.84 - 1.89)	0.222

Participant characteristics		n	Unadjusted relative risk (95% CI)	P-value	Adjusted relative risk (95% CI) (complete case analysis, N=2,144)	P-value
	Healthcare assistant	104	2.19 (1.60 - 2.96)	**<0.001**	2.12 (1.51 - 2.95)	**<0.001**
	Other	69	1.27 (0.77 - 1.96)	0.310	1.21 (0.70 - 1.92)	0.459
Vaccination status (any dose) ‡	Vaccinated (any dose)	2,313	Ref.	-		
	Unvaccinated	30	2.17 (1.41 - 2.95)	**<0.001**		
Vaccine brand (N=2,341) ‡	Comirnaty	1,902	0.79 (0.66 - 0.95)	**0.010**		
	Vaxzevria	377	Ref.	-		
	Other	32	1.93 (1.27 - 2.67)	**<0.001**		
	None (unvaccinated)	30	1.81 (1.15 - 2.57)	**<0.001**		
Vaccine type (N=2,341) ‡	mRNA (Comirnaty, Spikevax)	1,929	0.78 (0.66 - 0.95)	**0.010**		
	Viral vector (Vaxzevria, Janssen)	380	Ref.	-		
	Heterologous doses †	2	†	-		
	None (unvaccinated)	30	1.78 (1.13 - 2.51)	**<0.001**		

CI = confidence interval, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2. **Bold** values indicate significant P-values.

* Seropositivity unknown for one subject.

† Excluded from analysis due to small number in category.

‡ Excluded from analysis due to small numbers in some categories and resulting multicollinearity in multivariable analysis.

¶ Third level education excludes Post-graduate (NFQ Levels Level 9-10)

Table 5b Association between risk factors and SARS-CoV-2 seropositivity, St James's Hospital, PRECISE 4, Ireland, November 2021 (N=1,777*)

Participant characteristics		n	Unadjusted relative risk (95% CI)	P-value	Adjusted relative risk (95% CI) (complete case analysis, N=1,614)	P-value
Age groups (years)	18-29	311	1.49 (1.11 - 1.76)	**<0.001**	1.59 (1.24 - 2.04)	**<0.001**
	30-39	432	0.93 (0.73 - 1.18)	0.540	1.05 (0.81 - 1.36)	0.703
	40-49	533	1.00 (0.80 - 1.25)	0.980	1.10 (0.81 - 1.40)	0.458
	≥ 50	501	Ref.	-	Ref.	-
Sex	Female	1,413	Ref.	-		
	Male	364	1.03 (0.84 - 1.25)	0.770		
Ethnicity ‡	Irish (white)	1,374	Ref.	-		
	Any other white background	116	1.25 (0.93 - 1.64)	0.120		
	Asian background	262	1.37 (1.11 - 1.67)	**<0.001**		
	African and other black background	23	1.53 (0.81 - 2.42)	0.120		
	Unknown	2	1.31 (0.25 - 3.02)	0.650		
Country of birth	Ireland	1,297	Ref.	-	Ref.	-
	Philippines	127	1.39 (1.03 - 1.81)	**0.020**	1.20 (0.87 - 1.59)	0.225
	India	120	1.39 (1.02 - 1.82)	**0.020**	1.27 (0.93 - 1.68)	0.117
	United Kingdom	71	1.02 (0.62 - 1.52)	0.940	1.12 (0.67 - 1.68)	0.614
	Other	162	1.39 (1.07 - 1.77)	**0.010**	1.16 (0.85 - 1.52)	0.308
Education (N=1,639)	Primary/secondary/third level¶	1,168	1.68 (1.35 - 2.13)	**<0.001**	1.48 (1.18 - 1.90)	**<0.001**
	Post-graduate	471	Ref.	-	Ref.	-
Role (N=1,749)	Administration	260	Ref.	-	Ref.	-
	Medical/dental	154	0.73 (0.47 - 1.10)	0.140	0.70 (0.44 - 1.08)	0.115
	Nursing/midwifery	709	1.22 (0.95 - 1.59)	0.130	1.08 (0.82 - 1.45)	0.601
	Allied health	396	0.83 (0.61 - 1.13)	0.220	0.78 (0.57 - 1.09)	0.146
	General support	109	1.19 (0.80 - 1.73)	0.370	1.04 (0.64 - 1.59)	0.868
	Healthcare assistant	79	1.93 (1.36 - 2.69)	**<0.001**	1.82 (1.24 - 2.60)	**0.001**
	Other	42	1.17 (0.63 - 1.95)	0.570	1.24 (0.64 - 2.11)	0.469

Participant characteristics		n	Unadjusted relative risk (95% CI)	P-value	Adjusted relative risk (95% CI) (complete case analysis, N=1,614)	P-value
Vaccination status (any dose) ‡	Vaccinated (any dose)	1,759	Ref.	-		
	Unvaccinated	18	3.05 (2.08 - 3.85)	**<0.001**		
Vaccine brand (1,776) ‡	Comirnaty	1,421	0.83 (0.69 - 1.03)	**<0.001**		
	Vaxzevria	322	Ref.	-		
	Other	15	2.22 (1.27 - 3.23)	**<0.001**		
	None (unvaccinated)	18	2.67 (1.77 - 3.59)	**<0.001**		
Vaccine type (1,776) ‡	mRNA (Comirnaty, Spikevax)	1,434	0.84 (0.69 - 1.03)	0.090		
	Viral vector (Vaxzevria, Janssen)	323	Ref.	-		
	Heterologous doses †	1	†	-		
	None (unvaccinated)	18	2.65 (1.76 - 3.56)	**<0.001**		

CI = confidence interval, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2. **Bold** values indicate significant P-values.

* Seropositivity unknown for one subject.

† Excluded from analysis due to small number in category.

‡ Excluded from analysis due to small numbers in some categories and resulting multicollinearity in multivariable analysis.

¶ Third level education excludes Post-graduate (NFQ Levels Level 9-10)

Table 5c Association between risk factors and SARS-CoV-2 seropositivity, University Hospital Galway, PRECISE 4, Ireland, November 2021 (N=566)

Participant characteristics		n	Unadjusted relative risk (95% CI)	P-value	Adjusted relative risk (95% CI) (complete case analysis, N=530)	P-value
Age groups (years)	18-29	74	1.09 (0.65 - 1.78)	0.730	1.23 (0.72 - 2.06)	0.410
	30-39	152	0.89 (0.57 - 1.38)	0.590	0.96 (0.58 -1.48)	0.765
	40-49	192	0.89 (0.59 - 1.35)	0.570	0.98 (0.64 - 1.52)	0.945
	≥ 50	148	Ref.	-	Ref.	-
Sex	Female	472	Ref.	-		
	Male	94	1.14 (0.74 - 1.67)	0.530		
Ethnicity	Irish (white)	476	Ref.			
	Any other white background	51	1.32 (0.77 - 2.04)	0.260		
	Asian background	25	0.96 (0.36 - 1.89)	0.920		
	African and other black background	8	*	-		
	Unknown	6	*	**-**		
Country of birth	Ireland	439	Ref.	-		
	Philippines	4	*	-		
	India	9	*	-		
	United Kingdom	36	0.66 (0.25 - 1.35)	0.330		
	Other	78	1.04 (0.63 - 1.59)	0.870		
Education (N=540)	Primary/secondary/third level[¶]	394	1.10 (0.76 -1.65)	0.630		
	Post-graduate	146	Ref.	-		
Role (N=557)	Administration	102	Ref.	-	Ref.	-
	Medical/dental	44	2.51 (1.24 - 5.17)	**0.010**	2.80 (1.29 - 6.39)	**0.010**
	Nursing/midwifery	213	2.12 (1.23 - 3.99)	**0.010**	2.47 (1.35 - 5.19)	**0.007**
	Allied health	121	1.12 (0.56 - 2.32)	0.740	1.26 (0.58 - 2.89)	0.567
	General support	25	2.38 (0.98 - 5.31)	**0.040**	2.92 (1.08 - 7.28)	**0.022**
	Healthcare assistant	25	3.40 (1.62 - 7.03)	**<0.001**	3.93 (1.73 - 9.04)	**<0.001**
	Other	27	1.89 (0.71 - 4.41)	0.160	1.83 (0.60 - 4.87)	0.242

Participant characteristics		n	Unadjusted relative risk (95% CI)	P-value	Adjusted relative risk (95% CI) (complete case analysis, N=530)	P-value
Vaccination status (any dose) †	Vaccinated (any dose)	554	Ref.	-		
	Unvaccinated	12	0.79 (0.14 - 2.08)	0.720		
Vaccine brand (N=565) †	Comirnaty	481	0.62 (0.41 - 1.00)	**0.030**		
	Vaxzevria	55	Ref.	-		
	Other	17	1.52 (0.74 - 2.81)	0.200		
	None (unvaccinated)	12	0.54 (0.09 - 1.56)	0.360		
Vaccine type (N=565) †	mRNA (Comirnaty, Spikevax)	495	0.59 (0.41 - 0.93)	**0.010**		
	Viral vector (Vaxzevria, Janssen)	57	Ref.	-		
	Heterologous doses *	1	*	-		
	None (unvaccinated)	12	0.50 (0.09 - 1.43)	0.300		

CI = confidence interval, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2. **Bold** values indicate significant P-values.

* Excluded from analysis due to small number in category.

† Excluded from analysis due to small numbers in some categories and resulting multicollinearity in multivariable analysis.

¶ Third level education excludes Post-graduate (NFQ Levels Level 9-10)

Antibody response and durability

Table 6 presents the breakdown of COVID-19 vaccination status, as well as received vaccine brands and vaccine types. Classified according to participant self-reported receipt of required vaccine doses at the time of study conduct, the majority (97.7%) had completed a primary series of vaccination. Of those, 1,884 (82.3%) had received the Comirnaty vaccine, with a higher proportion in UHG compared to SJH. In SJH, almost one fifth of the participating HCW had received the Vaxzevria vaccine or a viral vector (Vaxzevria or Janssen) COVID-19 vaccine. Independent of partially or fully completed primary series of vaccination, all those vaccinated with any vaccine dose had detectable anti-S antibodies (results not presented). Fifty percent of those unvaccinated had detectable anti-S antibodies. Paired serology results (n=2,208) revealed that no participants lost their anti-S antibody positivity between PRECISE 2 in April and PRECISE 4 in November 2021. Of those 2,208 participants, 1,829 (82.8%) had anti-S antibody tire levels >250 U/mL (i.e. upper limit of quantification for the commercial assay) in April 2020, compared to 1,880 (80.2%) in November 2021. Additional assessment of titre levels of anti-S antibodies over time was not conducted, as the median value (>250 U/mL) equalled the upper limit of quantification. In terms of changes in the anti-N antibody status over time, 8.8% (n=40) lost their anti-N positivity between April and November 2021. As presented in **Figure 1**, there was a significant decrease in anti-N titres for the paired sera cohort between the two study phases with median values of 23.4 COI (range 1–262, IQR 6.8–70.9) in April 2021 decreasing to median 10.9 COI (range 0.271–330, IQR 2.79–34.3, p<0.0001) in the intervening seven-months period (November 2021). By age group, those aged 50 years or older (n=107) had a significantly greater reduction in titres in comparison to those aged 30–39 years (n=92) (median reduction 13.15 vs. 4.365 COI, p=0.014), with no significant differences demonstrated amongst other age groups (**Figure 1**). Unvaccinated HCW (n=4) demonstrated greater reductions in titres in comparison to those vaccinated (n=407) (median decrease 37.2 vs. 9.13 COI, p=0.047), and HCW vaccinated with the Vaxzevria (formerly AstraZeneca) vaccine (n = 88) demonstrated greater titre reductions in comparison to those in receipt of the Comirnaty (formerly Pfizer) vaccine (n=304) (median decrease 12.97 vs. 7.13 COI, p=0.007). Amongst anti-N positive participants in April 2021, dates for positive PCR results were known for 62.6% (n=286). Of these, time since infection impacted reduction in anti-N titres, with those infected >18 months prior to the current study demonstrating significantly smaller reductions in titres in comparison to those infected more recent, at 12–18 months or 6–12 months prior to study sampling (median reduction 4.8 vs. 11.1 (p=0.01) and 25.7 (p<0.001) COI, respectively). No significant differences in titre reductions were observed for sex, ethnicity, education level or infection status (natural versus breakthrough infection) (**Figure 1**). Seventy percentage (n=12) of individuals with breakthrough infections between first and second COVID-19 vaccine dose and 72.9% (n=51) of individuals with breakthrough infections after second COVID-19 vaccine dose of a two-dose primary vaccination series demonstrated anti-N positivity. Nearly ten percent (n=45) increased their anti-N titre levels. Of these, where positive PCR dates were known (n=29), the median time between SARS-CoV-2 infection and PRECISE 4 study participation was 412 days (IQR 323–590).

Comparison of SARS-CoV-2 seroprevalence over time (%)

A comparison of the crude seroprevalence in PRECISE 2 and PRECISE 4 by participant characteristics and by hospital site is shown in **Table 7**. The seroprevalence for the total study

population increased by 5.4% from April to November 2021. In SJH, the seroprevalence increased by 3.1%, while the seroprevalence increased by 8.0% in UHG. The increase in seroprevalence in UHG became more pronounced with increase in age, while the highest increase was in the age group of 18-29 in SJH. In the ethnic group of Irish (white), which most participants belonged to, the increase was most pronounced in UHG with 9.8% compared to 3.1% in SJH. There were no HCW with primary level of education in UHG in either of the two PRECISE study phases. From secondary level of education, the percentage increase in seroprevalence was higher with lower educational level among all participants and in both hospital sites. By staff role in the total study population, the increase in seroprevalence was highest among other staff roles (14.6%) and HCA (10.3%). In SJH, the highest increase was similarly shown among other staff roles (18.8%) but followed by administrative staff (6.3%). HCA (19.0%) presented with the highest increase in UHG with similar level of increases in the remaining staff roles.

Table 6 COVID-19 vaccination status, vaccine type and brand received by study participants, PRECISE 4, Ireland, November 2021 (N=2,344)

Participant characteristics		St James's Hospital		University Hospital Galway		P-value*	Total	
		(N=1,778)		(N=566)			(N=2,344)	
		n	%	n	%		N	%
Vaccination status	Primary series of vaccination, completed †	1,741	97.9	549	97.0	0.090	2,290	97.7
	Primary series of vaccination, partially completed ‡	11	0.6	2	0.4		13	0.6
	Unvaccinated	18	1.0	12	2.1		30	1.3
	Unknown	8	0.4	3	0.5		11	0.5
Completed primary series of vaccination (n=2,290)								
Vaccine brand	Comirnaty	1,408	80.9	476	86.7	**<0.001**	1,884	82.3
	Vaxzevria	319	18.3	55	10.0		374	16.3
	Other	14	0.8	17	3.1		31	1.4
	Unknown	0	0.0	1	0.2		1	0.0
Vaccine type	mRNA (Comirnaty, Spikevax)	1,420	81.6	490	89.3	**<0.001**	1,910	83.4
	Viral vector (Vaxzevria, Janssen)	320	18.4	57	10.4		377	16.5
	Heterologous doses	1	0.1	1	0.2		2	0.1
	Unknown	0	0	1	0.2		1	0
Partially completed primary series of vaccination (n=13)								
Vaccine brand	Comirnaty	8	72.7	2	100.0	0.787	10	76.9
	Vaxzevria	1	9.1	0	0.0		1	7.7
	Other	1	9.1	0	0.0		1	7.7
	Unknown	1	9.1	0	0.0		1	7.7
Vaccine type	mRNA (Comirnaty, Spikevax)	9	81.8	2	100.0	1.000	11	84.6
	Viral vector (Vaxzevria, Janssen)	1	9.1	0	0.0		1	7.7
	Heterologous doses	1	9.1	0	0.0		1	7.7
	Unknown	0	0.0	0	0.0		0	0.0

* Calculated using the chi-square test. **Bold** values indicate significant P-values.

† Defined as ≥14 days after receipt of the second dose of a COVID-19 vaccine of a two-dose vaccination course or first COVID-19 vaccine dose if receiving the Janssen COVID-19 vaccine.

‡ Defined as ≥14 days after receipt of the first dose of a COVID-19 vaccine of a two-dose vaccination course

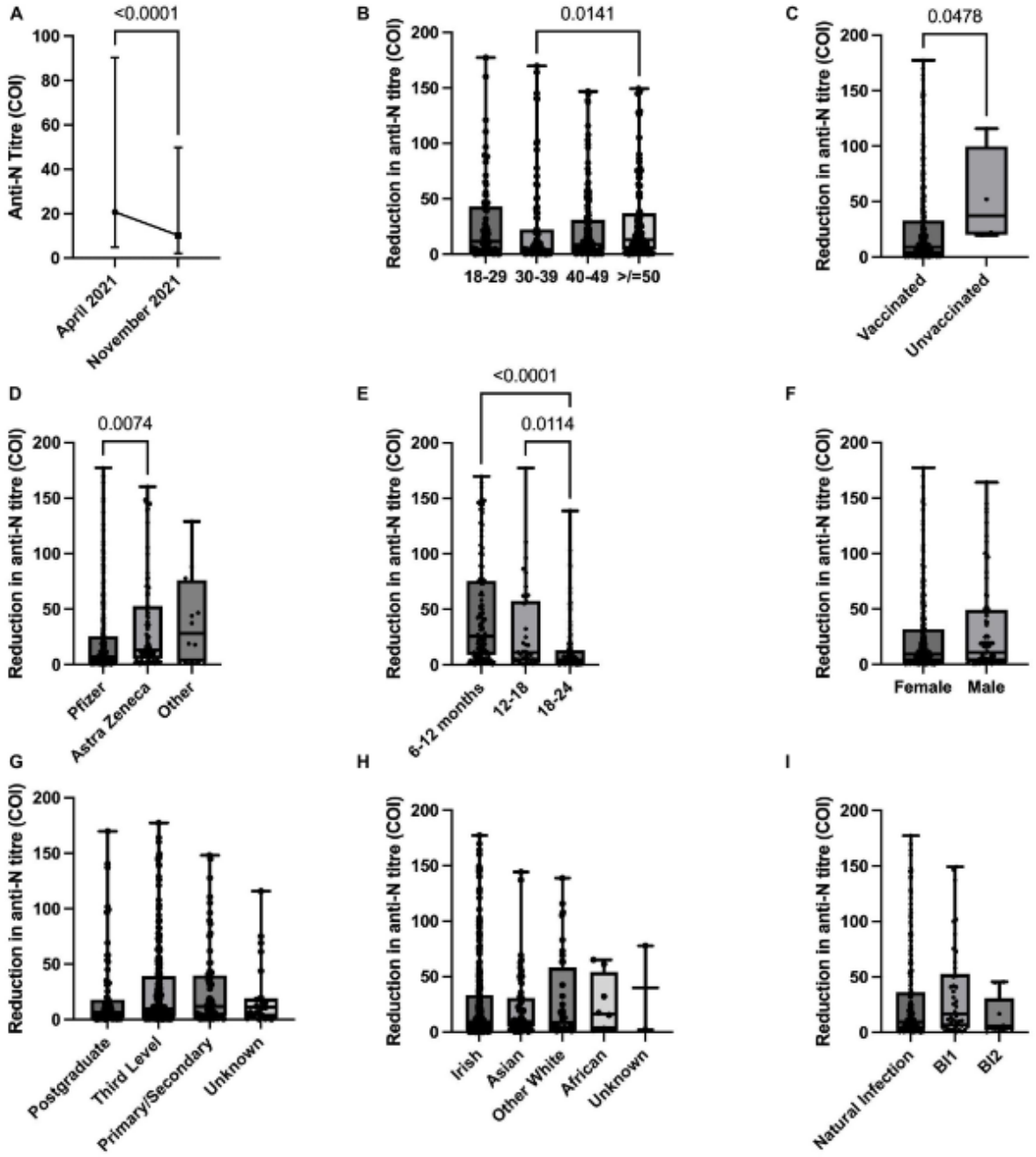

Figure 1 Anti-nucleocapsid (anti-N) titre dynamics over time between April 2021 (PRECISE 2) and November 2021 (PRECISE 4) (n=2,208). **(A)** Anti-N titre changes for paired sera cohort with positive anti-N in April 2021. (B-I) Reduction in anti-N titres by **(B)** age group, **(C)** vaccination status, **(D)** COVID-19 vaccine received, **(E)** time from reported positive SARS-CoV-2 PCR to PRECISE 4 study serology sampling (n=286), **(F)** sex, **(G)** education level, **(H)** ethnicity, **(I)** infection status: natural (pre-vaccine), breakthrough following one vaccine dose (BI1), breakthrough following second vaccine dose (BI2).

Table 7 Comparison of crude SARS-CoV-2 seroprevalence from April to November 2021, PRECISE 4, Ireland *

Participant characteristics		St James's Hospital			University Hospital Galway			Total		
		n (%) Apr-21	n (%) Nov-21	% change	n (%) Apr-21	n (%) Nov-21	% change	N (%) Apr-21	N (%) Nov-21	% change
Overall seroprevalence		623 (21)	429 (24)	3.1	275 (13)	119 (21)	8.0	898 (18)	548 (23)	5.4
Age groups (years)	18-29	159 (24)	100 (32)	8.2	90 (20)	18 (24)	4.3	249 (22)	118 (31)	8.6
	30-39	154 (20)	91 (21)	1.3	84 (15)	30 (20)	4.7	238 (18)	122 (21)	2.9
	40-49	157 (19)	122 (23)	3.9	51 (8.5)	38 (20)	11.3	208 (15)	160 (22)	7.1
	50-59	119 (21)	92 (23)	1.8	39 (10)	26 (22)	11.8	158 (17)	118 (23)	5.6
	Over 60	34 (23)	23 (24)	0.7	11 (8.4)	7 (24)	15.7	45 (16)	30 (24)	7.8
Sex	Female	471 (21)	339 (24)	3.0	198 (12)	97 (21)	8.6	669 (17)	436 (23)	6.1
	Male	152 (23)	90 (25)	1.7	77 (17)	22 (23)	6.4	229 (20)	112 (25)	4.5
Ethnicity	Irish (white)	401 (19)	304 (22)	3.1	194 (11)	99 (21)	9.8	595 (16)	403 (22)	5.8
	Any other white background	56 (22)	36 (31)	9.0	38 (17)	14 (28)	10.5	94 (20)	50 (30)	9.9
	Asian background	122 (26)	78 (30)	3.8	26 (20)	5 (20)	0.0	148 (25)	83 (29)	3.9
	African or other black background	29 (42)	10 (44)	1.5	10 (21)	0 (0)	-21.0	39 (33)	10 (32)	-0.7
	Other	15 (26)	-	-	7 (19)	-	-	22 (23)	-	-
	Unknown	-	1 (50)	-	-	1 (17)	-	-	2 (25)	-
Country of birth	Ireland	386 (19)	287 (22)	3.1	181 (11)	92 (21)	10.0	567 (16)	379 (22)	5.8
	Philippines	53 (27)	39 (31)	3.7	1 (6.3)	0 (0)	-6.3	54 (25)	39 (30)	4.8
	India	60 (27)	37 (31)	3.8	16 (24)	5 (56)	31.6	76 (26)	42 (33)	6.6
	United Kingdom	25 (19)	16 (23)	3.5	19 (12)	5 (14)	1.9	44 (15)	21 (20)	4.6
	Poland	8 (31)	2 (20)	-11.0	12 (20)	7 (30)	10.4	20 (24)	9 (27)	3.3
	USA	4 (19)	2 (20)	1.0	7 (21)	1 (11)	-9.9	11 (20)	2 (14)	-5.7
	Other	57 (28)	47 (32)	4.0	39 (28)	9 (20)	-8.4	92 (25)	56 (29)	4.0
Education	Primary	7 (35)	2 (67)	31.7	0 (0)	0 (0)	0.0	7 (32)	2 (67)	34.7
	Secondary	105 (26)	63 (33)	6.8	28 (14)	15 (28)	14.3	133 (22)	78 (32)	9.8

Participant characteristics		St James's Hospital			University Hospital Galway			Total		
		n (%) Apr-21	n (%) Nov-21	% change	n (%) Apr-21	n (%) Nov-21	% change	N (%) Apr-21	N (%) Nov-21	% change
	Third level†	301 (24)	252 (26)	1.9	133 (14)	68 (20)	5.9	434 (19)	320 (24)	5.4
	Post-graduate	210 (17)	76 (16)	-0.9	114 (12)	28 (19)	7.2	324 (15)	104 (17)	1.9
	Missing	-	36 (26)	-	-	8 (31)	-	-	44 (27)	-
Role	Administration	64 (16)	58 (22)	6.3	21 (7.7)	12 (12)	4.1	85 (13)	70 (19)	6.3
	Medical/dental	55 (15)	25 (16)	1.2	61 (17)	12 (30)	12.5	116 (6)	38 (19)	3.2
	Nursing/midwifery	281 (26)	193 (27)	1.2	114 (14)	53 (25)	10.9	395 (21)	246 (27)	5.7
	Allied health	89 (15)	73 (18)	3.4	29 (6.7)	16 (13)	6.5	118 (11)	89 (17)	6.2
	General support	60 (25)	29 (27)	1.6	21 (17)	7 (28)	11.0	81 (22)	36 (27)	4.9
	Healthcare assistant	70 (39)	34 (43)	4.0	21 (21)	10 (40)	19.0	93 (32)	44 (42)	10.3
	Other	4 (7.4)	11 (26)	18.8	6 (12)	6 (22)	10.2	10 (10)	17 (25)	14.6
	Missing	-	6 (21)	-	-	2 (22)	-	-	8 (22)	-

* Blank fields relate to inconsistency in categories between April 2021 (PRECISE 2) and November 2021 (PRECISE 4)

† Third level education excludes Post-graduate (NFQ Levels Level 9-10)

ACE2-RBD binding assay: antibody neutralisation

Demographics and vaccination status

Demographic, COVID-19 and vaccine related characteristics for the ACE2-RBD binding inhibition assay sub-cohort are presented in **Table 8**. Ninety samples were tested using the *in vitro* ACE2RBD binding inhibition ELISA. Age, sex, ethnicity, country of birth, education, and vaccine type received did not vary significantly from the total PRECISE 4 study population, although differences in job role and vaccination status proportions were noted.

Table 8 Participant characteristics in ACE2-RBD binding sub-cohort in comparison to total study population, PRECISE 4, Ireland, November 2021

Participant characteristics		Total study population (N=2,344)		ACE2 Binding sub-cohort (N=90)		P-value*
		N	%	N	%	
Age (years)	Mean (SD)	41.9 (11.0)		44.3 (11.4)		
	Median (IQR)	43 (33 – 50)		45 (34 – 53)		
Age groups (years)	18-29	385	16.4	13	14.4	0.15
	30-39	585	25.0	19	21.1	
	40-49	725	30.9	22	24.4	
	50-59	523	22.3	29	32.2	
	Over 60	126	5.4	7	7.8	
Sex	Female	1,886	80.5	71	78.9	0.7
	Male	458	19.5	19	21.1	
Ethnicity	Irish (white)	1,850	78.9	69	76.7	0.9
	Any other white background	167	7.1	6	6.7	
	Asian background	288	12.3	13	14.4	
	African and other black background	31	1.3	2	2.2	
	Unknown	8	0.3	0	0	
Country of birth	Ireland	1,736	74.0	64	71.1	0.9
	Philippines	131	5.6	6	6.7	
	India	129	5.5	7	7.8	
	United Kingdom	107	4.6	4	4.4	
	Other	241	10.3	9	10	
Education	Primary	3	0.1	0	0	0.2
	Secondary	245	10.5	16	17.8	
	Third level†	1,314	56.1	50	55.6	
	Post-graduate	617	26.3	20	22.2	
	Missing	165	7.0	4	4.4	
Role	Administration	362	15.4	14	15.6	
	Medical/dental	198	8.5	5	5.6	
	Nursing/midwifery	922	39.3	36	40	
	Allied health	517	22.1	12	13.3	

Participant characteristics		Total study population (N=2,344)		ACE2 Binding sub-cohort (N=90)		P-value*
		N	%	N	%	
	General support	135	5.8	14	15.6	**<0.001**
	Healthcare assistant	104	4.4	8	8.9	
	Other	69	2.9	0	0	
	Missing	37	1.6	1	1.1	
Vaccination status	Vaccinated (any dose)	2,313	98.7	82	91.1	**<0.001**
	Unvaccinated	30	1.2	8	8.9	
	Unknown	3	0.1	0	0	
	mRNA (Comirnaty, Spikevax) †	1,929	82.3	70	85.4	0.6
	Viral vector (Vaxzevria, Janssen) †	380	16.2	11	13.4	

IQR = interquartile range, SD = standard deviation

* Calculated using the chi-squared test. **Bold** values indicate significant P-values.

†Third level education excludes Post-graduate (NFQ Levels Level 9-10)

‡Comirnaty (formerly known as Pfizer), Spikevax (formerly known as Moderna), Vaxzenvria (formerly known as AstraZeneca)

ACE2-RBD binding inhibition

The extent of ACE2-RBD binding inhibition varied significantly by SARS-CoV-2 antibody status (**Figure 2**). The median ACE2-RBD inhibition demonstrated in anti-N positive individuals was 19.4% (mean 28.9%, range 1–91%, n=57) compared to 3.1% (mean 6%, range 0–25%, n=32) in those anti-N negative (p<0.0001) (**Figure 2**). For those anti-N positive, vaccinated participants demonstrated the highest levels of ACE2-RBD binding inhibition with median 20.9% (mean 30.5%, range 0–91, n=53) compared to those unvaccinated with median 5.3% (mean 7.3%, range 0.5–18.2, n=4). Where a positive PCR test was recorded for these participants, the median time from PCR to study sampling was 379.5 days (IQR 307–590, n=34). When assessed according to anti-N antibody status (anti-N positive or anti-N negative), ACE2-RBD protein-protein inhibition did not vary significantly by age, sex or vaccine type (Comirnaty or Vaxzevria).

The median ACE2-RBD binding inhibition was 13% in natural infection (mean 24.3%, range 0–87.8, n=30), a median of 415 days from infection to sampling (range 22–623 days, IQR 309.25–591.5), and higher in those with vaccination plus natural infection (median 16.2%, mean 25.8%, range 0–87.8%, n=27) compared to those with natural infection alone (7.0, 11.5, and 13% respectively, n=3). ACE2-RBD binding inhibition was median 21.7% in those with breakthrough infection (mean 16.1%, n=3) following the first COVID-19 vaccine dose (median 309 days from infection to sampling) and median 46.8% in those with infection following the second vaccine dose (n=4) (median 85 days from infection to sampling).

Participants in receipt of the Comirnaty vaccine, without evidence of prior SARS-CoV-2 infection, demonstrated a median ACE2-RBD inhibition of 1.7% (mean 4.5%, range 0–19.6%, n = 21), after a median 298 days from second COVID-19 vaccine dose (IQR 295–301).

Participants in receipt of the Vaxzevria vaccine, without evidence of prior SARS-CoV-2 infection, demonstrated ACE2-RBD inhibition of median 3.5% (mean 9.7%, range 0–25.6%, n = 5) after a median 196 days from second COVID-19 vaccine dose (IQR 190–196). ACE2-RBD inhibition in vaccine recipients with anti-N positivity was median 21.6% for Comirnaty vaccine recipients (mean 32.6%, n = 46) and median 12.8% for Vaxzevria vaccine recipients (mean 16.5%, n = 6).

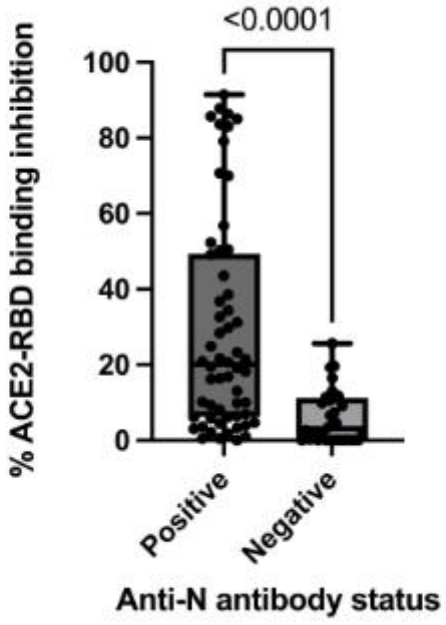

Figure 2 ACE2-RBD protein-protein binding inhibition stratified by participant anti-nucleocapsid antibody status for ACE2-RBD binding sub-cohort (n = 90).

Discussion

This report presents the findings of the PRECISE 4 study, a multi-site cross-sectional seroprevalence study of anti-SARS-CoV-2 antibodies in HCW in two hospital sites in Ireland, conducted in November 2021. This report complements the already published peer-reviewed paper on the PRECISE 4 study (23). By studying HCW over several study phases from PRECISE 1 in October 2020, PRECISE 2 in April 2021 and most recent PRECISE 4 in November 2021, this has allowed the assessment of risk factors in SARS-CoV-2 seropositivity and durability of antibody responses with the progression of the COVID-19 pandemic. The timing of the PRECISE 4 study immediately before the rollout of the COVID-19 booster vaccination programme was crucial to assess the serostatus of a high-risk population group while uninfluenced by booster dose receipt.

The PRECISE 4 participants were similar in demographics with previous PRECISE study phases and similar to European seroprevalence studies (5, 7, 36-38): predominated by females, belonging to the ethnic group (Irish) white and with a median age of approximately 40 years. The period of serology sampling was substantially longer in SJH than in UHG resulting in a higher proportion of participating HCW in SJH. The proportion of participants in each hospital is consequently skewed in comparison to the previous study phases, where participants were nearly evenly distributed between the two sites (5, 7). The higher proportion

in SJH is demonstrated in similarities with the results of the total study population. The study population was representative to the hospital workforce in terms of age, sex and professional categories, although the lower participation in UHG may have affected the representativeness of this site.

First cases of the Omicron VOC were identified in Ireland in week 47 (commencing 22nd November 2021), followed by a substantial increase in the COVID-19 incidence nationally in the following weeks (25). In same week 47, the reported COVID-19 incidence rate per 100,000 was higher in County Dublin than County Galway (759.8 vs 553.0 COVID-19 cases per 100,000). This persisted with exception of week 52 where County Galway temporarily exceeded County Dublin (25). The vast majority of the participating HCW in PRECISE 4 were vaccinated, and 97.7% of those vaccinated had completed a primary series of COVID-19 vaccination. In line with what has been reported by other authors, vaccine coverage for the COVID-19 primary series among HCW in Europe and the rest of the world in 2021 was generally high (39). The emergence of the Omicron variant in November 2021 raised concerns of the protective effect of previous SARS-CoV-2 infection or vaccine-induced immunity against breakthrough infections (40). Infection-induced immunity has been shown to persist up to one year during the Alpha and Delta pandemic waves (41), while immunity against Omicron in those previously infected seems to wane after a few months (42-44). In PRECISE 4, 14.7% with completed primary series of vaccination (before booster) had a SARS-CoV-2 breakthrough infection, which is similar to a previously published Swiss prospective multi-site cohort study among HCW conducted between June 2020 and March 2022 (45). This same study reported a substantial proportion of breakthrough infections (73.7%) among HCW who had already received a COVID-19 booster vaccine (and with either vaccine-induced or hybrid immunity), which further emphasises the significance of breakthrough infections during the Omicron-wave. SJH reported a higher proportion of breakthrough infections than UHG, which may underline the higher community incidence in County Dublin compared to County Galway, although extended duration of serology sampling in SJH limits the comparability between the hospital sites and to nationally reported COVID-19 rates.

The majority of vaccinated HCWs in PRECISE 4 received Comirnaty (81.1%) and Vaxzevria (16.1%). All vaccinated HCW had detectable anti-S antibodies, and no HCW in the paired serology lost their anti-S antibody status between April and November 2021. Persistence of anti-S positivity at 90% was reported by Dan and colleagues (46) at eight months after symptom onset, while the PRECISE 4 study demonstrated anti-S positivity at 100% over an extended time period in a highly vaccinated HCW population.

Nearly 9% of the participating HCW lost their anti-N positivity from April to November 2021. This likely reflects more dynamic anti-N antibody status, supported by other findings of reductions in anti-N positivity to 76-94% at eight months after infection (47, 48). It has also been demonstrated that a steep increase in anti-N antibodies within the first month post infection is followed by a decline thereafter, with an estimated half-life of 85 days and between 50% and 61% seronegativity between seven- and eight-months post infection, respectively (47-49).

The SARS-CoV-2 seroprevalence of the total study population was 23.4% in November 2021, which represented an increase from 18% in April 2021 and 10% in October 2020 (5, 7). This reflects the magnitude of the Delta pandemic wave in Ireland (beginning week 26 of 2021) (25) . HCW seroprevalence of SARS-CoV-2 was higher in SJH at 24.1%, compared to 20.0%

in UHG. Differences in seroprevalence between the two hospital sites have persisted since October 2020, when the PRECISE cross-sectional studies commenced. These are likely related to differences in local community incidence, social and demographic factors, and work practices. SARS-CoV-2 seroprevalence in October 2020 was six times higher than the community seroprevalence (7). This difference was less pronounced in November 2021, with estimated community seroprevalence (by anti-N positivity) between 7.9% and 12.1% at the time of the study conduct (50). This was equivalent to a two- or three-fold difference to the seroprevalence among the HCW participating in PRECISE 4. This demonstrates an increasing importance of community SARS-CoV-2 transmission with pandemic progression and a persistent HCW risk for infection. Reported community incidence has been consistently higher during the COVID-19 pandemic in the Dublin area, compared to the West of Ireland (25, 31). Evidence suggests that community incidence may be a risk factor for HCW seropositivity (51, 52), which combined with known community incidence in the areas of the PRECISE hospital sites could explain the higher seroprevalence in SJH compared to UHG throughout the PRECISE study phases (5, 7, 23).

SARS-CoV-2 seroprevalence from previous PRECISE studies in 2020 and 2021, reflected COVID-19 community incidence during the first and second pandemic waves in Ireland, which underlines the importance of multi-phase seroprevalence studies such as PRECISE to understand antibody dynamics over extended time. This has the potential to inform policies and strategies towards infection prevention and control (IPC) measures in healthcare settings. Other European studies in HCW conducted in 2021 have reported SARS-CoV-2 seroprevalences between 8.4% and 33.9% (38, 53-56). The varying timing of the studies, with differences in the magnitude and dynamics of the effect of the pandemic, constitutes a limitation to comparing findings. Notably, a prospective Spanish study found a similar steady increase in SARS-CoV-2 positivity in HCW from 11.8% in June 2020 to 28.4% in November 2021 (38).

HCA presented with the highest crude SARS-CoV-2 seroprevalence among all staff roles in November 2021, which had increased from 32% to 42% between April and November 2021. Being a HCA was further identified in multivariable analysis as constituting the highest risk of SARS-CoV-2 seropositivity, in consistency with previous PRECISE study phases (5, 7). Similar findings of increased risk associated with the HCA staff role have been reported in the literature (2-4). Whilst shortage of PPE is a known risk factor, there were no disruptive issues with PPE availability, training and/or use in the two hospital sites during the pandemic to our knowledge. Other factors outside the scope of this study may contribute to the HCA risk factor. The increase in SARS-CoV-2 seropositivity between April and November 2021 was more pronounced with lower level of education for the total population and SJH, while no HCW in UHG reported a primary level of education only. Level of education appeared as risk factor for SARS-CoV-2 seropositivity from the second study phase in April 2020, with a higher relative risk with lower educational level (5). HCW in SJH were similarly of increased risk with a primary/secondary/third level education level, compared to post-graduate. Lower socio-economic status has previously been correlated with an increased risk of COVID-19 infection and poor clinical outcomes. An increased risk for black ethnicity has also previously been reported (57). Belonging to the ethnic group of African or other black background doubled the risk of SARS-CoV-2 seroprevalence, compared to being Irish (white) in PRECISE 2 (5). Caution in interpretation is required, as a low proportion (1.3%) of the participating HCW in PRECISE 4 belonged to the African or other black background subgroup, of which

approximately one third were seropositive. Ethnicity were not assessed in multivariable analysis due to multicollinearity with country of birth. Percentage increase in SARS-CoV-2 seropositivity from April to November 2021 was in line with the identified risk factors associated with seropositivity. Understanding the high-risk groups in health and care settings is crucial for targeted messaging and educational strategies to address risk of transmission.

The findings regarding ACE 2-RBD binding inhibition have been elaborately discussed in the published PRECISE 4 paper by McGrath et al. (2023) (23). Higher levels of RBD-ACE2 binding inhibition (median 20.9%) than those with either vaccination or infection alone were demonstrated in participants with both vaccination and previous SARS-CoV-2 infection (evidenced by anti-N positivity), suggesting a greater degree of humoral immune protection in this subgroup. RBD-ACE2 binding inhibition of mean 4.5 and 9.7% was demonstrated for participants in receipt of Comirnaty and Vaxzevria vaccination alone, a median 298 days and 196 days post second dose, respectively. This is comparable to the inhibition seen in unvaccinated individuals with natural infection alone, determined via anti-N positivity, at 7.3% (n = 4). While inhibition was increased in the Vaxzevria group compared to the Comirnaty group, this is influenced by time interval from primary vaccination schedule completion to serology sampling (as above) and did not show statistically significant differences.

These observations are supported by findings in vaccine effectiveness (VE) studies, with Hall et al. demonstrating VE consistently higher than 90% in those with infection plus vaccination up to 18 months following infection, while infection-acquired immunity waned in individuals with natural infection alone after 1 year (20). The degree of RBD-ACE2 binding inhibition required to mediate this increased protection in vaccinated/previously infected individuals is unclear and is likely one factor of many in the functional immune protection conferred by vaccination. Additionally, it is noted that modelling data suggests that the neutralisation level required for protection from severe infection is six-fold lower than the level required to protect from any symptomatic infection (58) and thus the lower RBD-ACE2 inhibition levels demonstrated in the vaccination alone/infection alone subgroups may still provide a degree of protection over time. The reported magnitude of NAb titre reductions over time varies, with some studies demonstrating a median decrease of 34.8% three months post vaccination (59). Our findings support a reduction in NAbs over time, with ACE2-RBD inhibition of median 46.8% demonstrated in participants with infection following the second vaccine dose a median 85 days prior to sampling, in contrast to 16.2% in individuals with natural infection and vaccination a median 415 days prior to sampling. Given the small numbers involved in these sub-groups in the current study, it is difficult to determine statistical significance from this finding but it should be considered a signal warranting further investigation.

The emergence of VOC has resulted in reductions in VE (60)and vaccine-derived NAbs (61). Reports have demonstrated significant NAb titre reductions against the Omicron variant in individuals in receipt of two doses of mRNA vaccine or with a history of natural infection alone without vaccination (62). Booster vaccine dosing with mRNA vaccines mitigates this antibody reduction with significant increases in titres demonstrated in both previously infected individuals and those in receipt of vaccination alone (61, 62). The variations in participant antibody neutralisation capacity demonstrated in PRECISE 4, assessed by infection/vaccination status, support findings identifying at-risk groups for VOC infection and add to the evidence and rationale for additional vaccine doses. As population-wide vaccine programmes become increasingly challenging logistically, a more targeted approach may be preferable with higher risk groups as identified earlier, being prioritised for further vaccinations.

Limitations

This present study has a number of limitations. Although a thorough validation exercise of reported dates pre-empted the data analysis, self-reported dates of positive PCR test results and COVID-19 vaccine dates led to some uncertainty regarding their reliability. When feasible, it is generally preferred not to use self-reported dates in vaccine studies or other studies in which the data analysis heavily relies on their accuracy. Further to this, the data collection took place during the Delta pandemic wave, characterised by its large magnitude and inevitable impact on the experienced work pressure in healthcare settings, representing the frontline response to an international health threat. The majority of the participants had taken part in the previous study phase, which enabled an assessment of the changes in seropositivity and antibody response over time. However, the participation rate was substantially lower for PRECISE 4 compared to PRECISE 2 despite recruitment efforts via several platforms. This may be explained by a natural declining interest in COVID-19 research as the pandemic progresses together with likely work fatigue.

The fact that few participants were born in countries other than Ireland may be explained by the lack of translation of the study material into languages other than English. This would not allow anyone unfamiliar with English or those with lower professional need for English (e.g. certain non-patient contact occupational roles) to participate, although they may likely constitute a considerable risk group. Acknowledging the time pressure to initiate data collection before the rollout of the COVID-19 booster vaccination programme, multilanguage information and targeted communication and identification of non-English speaking HCW through the appropriate messaging avenues should be considered for future studies to limit potential selection bias.

Multicollinearity in the multivariable analysis did not allow the assessment of all variables for inclusion, and aRR could not be determined for e.g. ethnicity which has previously been associated with an increased risk of SARS-CoV-2 infection. Data collection in a highly vaccinated population limited the risk assessment to a simpler categorisation of being vaccinated (with any dose) or unvaccinated, rather than allowing a statistical analysis of the risk of SARS-CoV-2 seropositivity by partially completed and completed vaccination primary series. The upper limit of quantification for the commercial Roche Elecsys-S Anti-SARS-CoV-2 assay prevented an assessment of titre levels of anti-S antibodies over time as the median equalled the upper limit of 250 U/mL.

Conclusion

Healthcare workers (HCW) remain a high-risk population for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral transmission. Increase in SARS-CoV-2 seroprevalence from April to November 2021 reflected the magnitude of the Delta pandemic wave in Ireland, with persistently higher seroprevalence in one hospital site situated in a higher density area with higher community incidence. These findings highlight community incidence as a substantial risk to HCW.

Factors associated with SARS-CoV-2 seropositivity was dependent on age, country of birth, educational level and staff role of HCW. Infection prevention and control (IPC) measures should consider the increasing evidence burden on high-risk groups in healthcare settings,

and this should inform targeted messaging and educational strategies to address increased transmission risks.

While all vaccinated HCW maintained their anti-spike (S) positivity prior to COVID-19 booster vaccination, anti-nucleocapsid (N) antibody status demonstrated more dynamic changes over time. With waning immunity over time (both for vaccine induced and/or natural immunity) and increasing logistical issues pertaining to vaccination at population-level, the current study may inform targeted vaccination and educational strategies towards high-risk population groups, including HCW most at risk.

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Appendix A PRECISE 4 Participant Information Leaflet

PRECISE Study
Antibody Test for COVID-19

PRECISE 4 participant information leaflet, 08/10/2021

Prevalence of COVID-19 Antibodies in Irish Healthcare Workers

If you have previously participated in the research study PRECISE 2, in either St James's Hospital or Galway University Hospital, we are inviting you to take part in PRECISE 4, a research study looking at levels of COVID-19 antibodies in healthcare workers. This study is a national study which has been advocated for by the National Public Health Emergency Team (NPHET), Department of Health, and coordinated by the Health Service Executive (HSE) as part of the national response to the COVID-19 pandemic. St. James' Hospital and University Hospital Galway have been selected as the testing sites. Participation in the study is voluntary meaning that you only need to take part if you want to. This leaflet provides information about the study.

What is COVID-19 (coronavirus)?

COVID-19 infection caused by SARS-CoV2 virus is a new pandemic infection that can affect your lungs and general health and is causing illness in Ireland and spreading rapidly around the world. It is caused by a virus called coronavirus. Most people who get infected only have a mild illness. However, it can cause a more severe illness and death, particularly in people who are older or have other illnesses.

What is the study and why is it being done?

SARS-CoV2 is a new virus and there are many things we don't know about it. Up to now, we have been testing people in Ireland who are ill and we have been counting the number of cases of infection. The results of the SCOPI study (Study to Investigate COVID-19 Infection in People Living in Ireland) have shown coronavirus antibodies among the population of Dublin to be estimated at 3.1% and among the population of Sligo to be estimated at 0.6%. Our study is specifically aimed at healthcare workers; we want to find out how widely COVID-19 has spread in healthcare workers living in Ireland. This study aims to find out how widely COVID-19 has spread in healthcare workers living in Ireland. When a person becomes infected with a new virus, their body produces a response – called antibodies - to the virus.

These antibodies can usually 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, in most cases, if the person has been infected at some stage with the virus that causes COVID-19 infection. The study will help in making decisions about how to control the spread of the infection, how to understand the risks of acquiring infection either in work or outside of work and how to make the hospital a safer working environment. It may also be valuable for informing us how to evaluate vaccine responsiveness and effectiveness.

Who is doing this study?

This study is being coordinated by the HSE, and undertaken by St. James' Hospital and Galway University Hospital. The study will be coordinated by a research team from all three centres.

How will it be done?

All staff members who participated in PRECISE 2 in April 2021, working in St. James' Hospital, Dublin, and University Hospital Galway are being invited to participate in the study. Participants are invited from all departments in the hospitals. People who are taking part in the study will be asked to answer a short questionnaire and to give a blood sample in their hospital. Participation in this study is voluntary and if you would like to take part, we will ask you to indicate your consent. We did the same study in October 2020 and April 2021. We are repeating it to see if the amount of healthcare workers with antibodies has changed a lot. If you took part in the previous studies, we will ask your permission to link your results, so that we can see if your result has changed. If you took part in the previous studies but do not want us to link your results you can still take part again.

How does the vaccine affect the study?

The roll-out of booster vaccination has added an extra component to this study. We will also be able to measure antibody response to vaccination in this study. You are invited to participate whether you have been vaccinated or not.

If you have already had COVID-19 infection can you still take part?

Yes. If you have already had COVID-19 infection, we would still like you to take part in the study. If you are still isolating because of your illness, we will not ask you for a blood test but would still ask you to answer the questionnaire.

Can everyone who is asked, take part in the blood testing part of the study?

Unfortunately, anyone who has been advised to 'cocoon', anyone who has suspected or confirmed COVID-19 at the moment, and anyone who is restricting their movements because they are a close contact of COVID-19 case, will be excluded from the blood testing part of the study. This is because travelling to the testing centre may be a risk to their health or the health of others. They can still contribute to the questionnaire part of the study.

Any healthcare worker who has symptoms should not come to work and should contact the Occupational health department at their hospital site.

What am I being asked to do?

The study involves filling out a short questionnaire and taking a blood test. If you are receiving the booster dose of the COVID-19 vaccine, we will take the sample before you receive the dose. If you agree to take part in the study, we will ask you to do the following:

1. Sign a consent form.
2. Following completion of the consent form you will be asked to complete a brief questionnaire (5-10 minutes)
3. Provide a blood sample. The testing will be carried out in the hospital in which you work. We will make sure that physical distancing is in place at this location, and face-masks will be obligatory. The blood sample will be taken by trained personnel.

What information will you collect?

We will record the following information about you: Name, contact details, date of birth, sex, occupation, country of birth, medical history and who shares your household. We will ask you questions about COVID-19 illness, like whether you have been diagnosed with the condition, have had a swab taken (whether positive or negative) or have had symptoms that might have been COVID-19 illness, or if you had contact with a known or suspected case of COVID-19. We will also collect information about previous COVID-19 vaccination, including which vaccine you received, and how many doses you have received.

How will my information be used and stored?

The only people who will be able to see your information will be members of the research team at the site where you work. These will be the principal investigator, the lead investigator and the data manager. Your data will always be managed confidentially. Your data and questionnaire answers will be held in a secure online database, or securely in written format where applicable. Only personal data necessary to carry out the study will be processed and stored in the database. Your personal data will not be disclosed outside of the study group and will not be linked to your name on the hospital laboratory system. Your records in the study will not be linked with any other records that may be held in other databases or registers. Data will be only be collected and used in this study to the extent needed to achieve the aims. Data will be processed for the purposes of public interest, in the area of public health, for scientific research, in accordance with applicable data protection legislation. All groups involved in the study operate in compliance with the General Data Protection Regulation (EU) 2016/679 and with the Data Protection Act 2018 and are accredited for information security ISO 27001. All staff and researchers involved in the study have had data protection training. It will not be possible for any participant in the study to be identified in reports that are produced from the study. Your personal data will only be held for as long as it is needed for this study and for not more than five years. After that time, your name, address, and anything else that could identify you will be deleted. The plan for the study has been approved by the National

Research Ethics Committee set up by the government to approve research on COVID-19. For further information on this, please see the website: https://www.hrb.ie/news/covid-19-coronavirus/coronavirus-news/article/new-nationalethics-committee-for-covid-19-research/

What are my rights?

All participants have the following rights in relation to data:

- Right to access data held
- Right to restrict the use of the data held
- Right to correct inaccuracies
- Right to have information deleted
- Right to object to profiling
- Right to complain to the supervisory authority (Data Protection Commission)

You can exercise these rights by contacting the PI or DPO, contacts listed in this leaflet.

What will happen to my blood sample?

Your blood sample will be tested for the antibodies to COVID-19 virus. We will test for two types of antibodies, one that is produced in response to infection with COVID-19 as we did in October, and another that is produced in response to vaccination. We will also perform a test to look at the cells of your immune system. Your blood sample will be stored for a period of two years after completion of the study and will then be destroyed. During this time, if you agreed to it on the consent form, we might contact you about using your sample for other closely related studies that will help us to continue to improve our understanding of this virus. Any additional studies will undergo ethics review and approval.

Will I get the result of my blood test?

When signing the consent form, you will be asked whether or not you wish to receive the result of your antibody blood test. If you wish to receive it, we will text you your result, and a brief explanation of what the result may mean. As this is a new disease the tests for it are also new. It is still not clear exactly what the results mean for people themselves. This test is not a test to diagnose current COVID-19 infection in an individual – it is designed to help us to estimate the number of infections at population level. The antibody blood result should not be used to make a decision on whether or not to receive a booster vaccine dose. If antibodies to the COVID-19 virus are not found in your blood, it may mean that:

• You may have never been infected with COVID-19 – this is the most likely explanation

• You have been infected but had a mild infection and have a level of antibody in your blood that the test we are using is not able to detect. If antibodies to the COVID-19 virus are found in your blood, it may be a sign that you were infected with the COVID-19 virus at some time. However, we do not know now whether this will give you protection against getting the infection again. The reason we do not know is because this is a new disease and the tests for it are also new. **So, your result cannot be taken to mean that you are immune to COVID-19. It is very important that you continue to follow all the physical distancing and hygiene measures recommended by the government, and supported by your local Infection Prevention and Control team, and follow all advice in relation to PPE use.**

Sometimes the test can show a positive result even though you do not actually have the antibody. This may be more likely to happen if you have recently had infection with another virus or if you are pregnant or in certain other situations. This possibility will be discussed with you if you are phoned with a positive test result.

Sometimes, the test result can be inconclusive, meaning that the laboratory is unable to give a clear result one way or the other. You may be offered another test to try to see if that gives a clear result. We will phone you if this is the case.

If you have been vaccinated against COVID-19 we may be able to see this in your antibody result.

If you have been vaccinated against COVID-19 but the test indicates that antibody is not detected, this does not necessarily mean that the vaccine is not working the way it should. There are other mechanisms of immunity that we are not testing for in this study.

The antibody blood result should not be used to influence a decision on receiving further COVID-19 vaccine doses.

Will anyone else get the result of my blood test?

Only you will receive the result of your individual antibody blood test. The study team will not inform your GP or Occupational Health of your result. Your result will not be attached to any of your records in the hospital, either in HR or your medical records.

How will the results of the study be used?

Even though we can't be sure of the meaning of the results for each person, the results will give us (1) very useful information about the transmission of the virus in the hospital setting, (2) will allow us to assess the risk of acquisition of infection over time (3) will inform our understanding of measurable sustained immune response over time and (4) will inform you and us about the response to the vaccine. Anonymised results (that is, without the possibility of identifying anyone) will be shared with the Department of Health COVID-19 team and HSE to help with making decisions about how to control the spread of the virus. A report with the overall results of the study will be made public on the HPSC website once we have analysed all the information. Results will also be published in a scientific peer-reviewed journal. These results will not identify any individuals.

What if I do not want to take part?

Participation in this study is voluntary. If you do not wish to take part, neither your care nor your work will be affected by this decision in any way now or in the future. If you agree to take part in the study, you have the right to withdraw your consent at any time, by contacting the local lead at your study site listed below.

St. James' Hospital www.stjames.ie
Professor Colm Bergin, Consultant Physician in Infectious Diseases
Dr. Jonathan McGrath, Specialist Registrar in Infectious Diseases

Data Protection officer, SJH
Galway University Hospital www.saolta.ie
Dr. Catherine Fleming, Consultant Physician in Infectious Diseases
Data Protection Officer, GUH

Appendix B PRECISE 4 Patient information Questionnaire

PRECISE 4: Patient information Questionnaire

First name: _____

Surname: _____

Date of Birth: _____

Gender: _____

Country of Birth: _____

Ethnicity: Please tick one box:

- ☐ Irish
- ☐ Irish Traveller
- ☐ Other White background
- ☐ African
- ☐ Other Black background
- ☐ Chinese
- ☐ Other Asian Background

In what role do you currently work? Please tick one:

- ☐ Admin
- ☐ Allied health
- ☐ Ambulance
- ☐ Catering
- ☐ Chaplain
- ☐ Cleaning
- ☐ Dental
- ☐ Doctor
- ☐ Driver
- ☐ Education
- ☐ HCA
- ☐ IT
- ☐ Laboratory
- ☐ Maintenance
- ☐ Midwifery
- ☐ Nursing
- ☐ Porter
- ☐ Research
- ☐ Security
- ☐ Technician

PRECISE 4: Patient information Questionnaire

Level of Education: _____

Did you take part in the PRECISE Study in April 2021? _____

Have you received a COVID vaccine? _____

Which vaccine did you receive? _____

Have you received all required doses of the vaccine? _____

What was the date you received your second dose of vaccine? _____

(Only dose if J&J) _____

Can we access your vaccination records if needed? _____

Have you had confirmed infection (by PCR) with COVID-19 before receiving the first dose of vaccine? _____

Have you had confirmed infection (by PCR) with COVID-19 since being fully vaccinated? _____

If you received a vaccine that required two doses. Have you had confirmed infection (by PCR) with COVID-19 between 1 and 2nd dose of the vaccine? _____

What was the date of this positive PCR? _____

Are you happy to be contacted via text message with your results? _____

What is your phone number? _____

Please re-enter your phone number _____