Correlation between a commercial SARS CoV 2 anti-spike RBD assay and neutralising antibody in the assessment of a functional antibody response in samples collected during a seroprevalence study in Ireland

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Abstract

Background

This prospective surveillance study investigated the correlation between a commercial SARS-CoV-2 anti-spike RBD IgG assay used for routine serosurveillance and a commercial assay specific for neutralising antibodies, to determine if serosurveillance testing could provide estimates of potential clinical protective immunity in an Irish population.

Methods

Residual anonymised sera were collected from adults as part of the Irish National Serosurveillance Programme (NSP: https://www.hpsc.ie/a-z/nationalserosurveillanceprogramme/) in January and February 2023. Samples (n=287) sourced from general practice and blood donors, were analysed using the Abbott quantitative anti-Spike IgG assay (S) and were further characterised using the Roche anti-Nucleocapsid antibody assay (N). Neutralisation activity was determined using the GenScript neutralisation antibody detection assay (cPass). Based upon the cPass assay results, selected sera were subsequently analysed using an “in vitro” infectious virus neutralisation assay (TCID50). Statistical analysis (e.g. Spearman’s correlation, modelling) was performed in R, RStudio. In addition, TCID50 testing was performed comparing ancestral virus and omicron BA.5 variant on selected samples.

Results

Comparison of Abbott quantitative anti-spike IgG levels with percentage inhibition results generated using the cPass assay demonstrated an initial linear response with significant correlation (Spearman’s rho = 0.6) which plateaued with levels of neutralisation above 95%. This trend was not impacted by age, sex, source of sample, infection or vaccination history (S+N+ or S+N-). The breakpoint between the linear increase and plateau occurred at 417 BAU/ml anti-spike antibody as determined by modelling. Testing using TCID50 assays, against ancestral and omicron BA.5 also confirmed high levels of viral neutralisation in serum samples.

Conclusions

This study demonstrated a good correlation between anti-spike RBD antibody levels detected using a commercial assay in routine use and specific neutralising antibody in an Irish cohort. It was observed that specimens with high levels of anti-spike IgG collected from individuals over 65 years and from those without anti-nucleocapsid antibody showed significant neutralisation activity. This work will add to the international evidence base regarding the assessment of the humoral response associated with clinical protection against SARS-CoV-2 and will inform ongoing evaluation of vaccination strategies and other public health interventions.

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Background

The National Serosurveillance Programme (NSP) is led by the HPSC Seroepidemiology Unit (SEU), working in partnership with the University College Dublin (UCD) National Virus Reference Laboratory (NVRL) Serosurveillance Unit, the acute-hospital Laboratory Surveillance Network (LSN) and the Irish Blood Transfusion Service (IBTS). It is overseen by a national multi-disciplinary and multi-sectoral Steering Committee. The NSP conducts surveillance of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) seroprevalence in the adult population using residual sera sourced from general practice, collected by the LSN, and residual sera from blood donors collected by the IBTS. The aim of SARS-CoV-2 serosurveillance is to provide up to date estimates of SARS-CoV-2 seroprevalence, including quantitative assessment of antibody levels by age and sex. Timely and accurate population seroprevalence data are critical to inform proactive, targeted public health interventions, vaccination policy, and treatment interventions.

Whilst the presence of certain SARS-CoV-2 antibodies can distinguish to some extent between past infection and vaccination, antibody measurement cannot at this point determine an antibody level which correlates with clinical protection. A subset of antibodies (called functional antibodies) are associated with viral neutralisation and inhibition and may prevent the serious clinical sequalae following infection. However, functional antibodies are not detected in routine serosurveillance and the methodologies used are unsuitable for large-scale testing. Direct assessment of functional antibodies requires specific facilities, equipment, and trained personnel that are not broadly available to most diagnostic laboratories. Antibody-mediated neutralisation of viruses is the direct inhibition of viral infectivity as a result of antibody binding to virus particles, preventing entry of the virus into cells, and thus blocking a non-redundant step in the viral replication cycle that precedes virally encoded transcription or synthesis (3)(4). As such, neutralising antibodies (NAbs) are crucial for vaccine-mediated protection against viral diseases, in addition to being markers of clinical protection following reinfection (3)(5).

The NSP Steering Committee raised the question as to whether the quantitative commercial available SARS-CoV-2 anti-RBD immunoassays that are used in the current serosurveillance studies in Ireland show correlation with functional antibody assays, thereby potentially allowing for estimates of an antibody level which may provide protection against clinical illness. Such estimates could support public health interventions, which may be developed through ongoing assessment of humoral protective immunity in the population, both in recovered patients and in vaccine recipients, and for the ongoing evaluation of vaccine strategies. There are also potential benefits at the individual level, including tailoring of medical advice (e.g. recommendation for booster vaccination) based on quantitative antibody level in combination with patient characteristics and clinical information.

In 2021, Jung et al., evaluated the correlation between the Abbott Architect SARS-CoV-2 IgG II Quant assay and the GenScript cPass™ SARS-CoV-2 Neutralisation Antibody assay, the neutralisation assay used in this study protocol. The study included 173 sera collected from 126 SARS-CoV-2 hospitalised patients and 151 pre-pandemic sera in

Korea. The assays showed good correlation (Spearman's rank-order correlation coefficient (rho) of 0.87 ($p<0.001$)). Limitations of this study included that few asymptomatic patients were included. Correlates by age, sex and SARS-CoV-2 variant were not investigated.

Lin et al., in 2022, using blood donor samples in Canada, conducted a correlation study using the Abbott Architect SARS-CoV-2 IgG II Quant assay and a culture-based neutralisation assay, and demonstrated that plasma samples with values approaching 10^4 BAU/mL showed strong evidence of functional neutralisation of SARS-CoV-2 Delta variants. The study included wild-type, alpha, beta, gamma, and delta SARS-CoV-2 variants but did not include omicron which was noted as a limitation of the study. Correlates by age and sex were not investigated.

Other correlation studies were identified that involved quantitative antibody tests other than the Abbott Architect SARS-CoV-2 IgG II Quant assay. In 2021 Dolscheid-Pommerich et al., demonstrated a strong positive relationship between anti-S1 IgG levels using the QuantiVac ELISA assay and neutralising antibody titres ($r_s = 0.819$, $p < 0.0001$). In 2021, Peterhoff et al., conducted a study demonstrating a strong correlation between antibody levels obtained using Euroimmun SARS-CoV-2-ELISA IgG test and neutralisation titres (given as IC50 values) (anti-RBD ($R2 = 0.8943$, Spearmen's $\rho = 0.965$, p value < 0.0001) and anti-StabS ($R2 = 0.9057$, Spearmen's $\rho = 0.964$, p value < 0.0001). In 2021, Šimánek et al., demonstrated that the neutralising effect is stronger with increasing age. Šimánek et al investigated correlation between five commercial SARS-CoV-2 qualitative/semi-quantitative immunoassays and a virus neutralisation test (VNT). In this study of non-hospitalised patients, elderly patients had a higher proportion of high neutralising titers when compared to young patients. In terms of assay suitability, those targeted against the spike protein showed higher correlation, with assays targeted against the nucleocapsid protein showing less correlation. A study by El-Ghitany et al., 2022, on 650 serum samples concluded that there was correlation between the quantitative Anti-SARS-CoV-2 QuantiVac ELISA (IgG) and the NeutraLISA neutralisation assay (both by EUORIMMUN). Other similar studies are available demonstrating correlation between various quantative anti-spike antibody tests and neutralisation tests (Salazar et al., 2020; Valdivia et al., 2021; Moscato et., 2021; Dinc et al., 2022, Tsuchiya et al., 2023).

Aim & Objectives of this Study

The aim of this study is to investigate correlation between the Abbott Architect SARS-CoV-2 IgG II Quant anti-RBD assay antibody levels and neutralising antibodies using GenScript Biotech C-Pass assay. This is to inform our understanding of the relationship between currently available quantitative antibody assay levels to determine a level of SARS CoV 2 antibody which provides protection against serious clinical illness in an Irish population.

Specific objectives

- To investigate if correlation is present between the Abbott Architect SARS-CoV-2 IgG II Quant quantitative SARS-CoV-2 antibody level and percentage neutralising ability, overall, and by **age group**, using sera sourced from both LSN adult hospitals and the IBTS,
- To investigate the ability of selected subset of sera to neutralise different **SARS-CoV-2 variants,**
- To report on and use the data from this study to help to advise on public health actions that may be required, including for example COVID-19 vaccination campaigns,
- To support assessment of antibody which may provide protection against serious clinical illness in an Irish population, to inform ongoing evaluation of vaccine strategies and other public health interventions

Methods

A group of subject matter experts were convened to oversee this study which was termed as the Functional Antibody Correlation Study or FACS. This group met regularly throughout the study to discuss the design of the study and results. The membership of this group can be seen in Appendix A. The group also provided regular updates to the National Steering Committee from September 2022 to Dec 2023. Routine serosurveillance testing and FACS testing (which was divided into two Phases) are described in this section.

Current serosurveillance Sampling

The NSP conducts systematic sampling of residual specimens from six acute hospital clinical chemistry laboratories within the LSN, and from IBTS clinics at regular intervals. Residual sera specimens are blood samples that were originally collected for clinical testing and are now due to be discarded. The residual samples are anonymised and then tested for antibodies to SARS-CoV-2 (S and N). Blood donor samples are tested on site in IBTS and in St James's Hospital. Samples from the acute hospital LSN are tested in the NVRL.

Samples for this prospective surveillance study were collected during January and February 2023 from two sources as follows:

- Specimens from adults (18+ years) collected as part of Cycle 10 of the Laboratory Surveillance Network (LSN) serosurveillance program for COVID-19. The LSN network comprised six participating acute hospital clinical chemistry laboratories: Letterkenny University Hospital, St. Vincent's University Hospital, University Hospital Limerick, Galway University Hospital, Beaumont Hospital and Tallaght University Hospital.
- Specimens from adults (18+ years) collected as part of epidemiological Week 3 and Week 5 routine serosurveillance program for COVID from Irish Blood Transfusion Service (IBTS). These specimens were collected from three fixed IBTS blood donation clinics in Ireland, two of which are in Dublin and one in Cork. Sequential sampling of blood donors aged 20-79 takes place until a target of 500 valid specimens is reached per sample week.

Routine serosurveillance anti-Spike and anti-Nucleotide antibody testing

Two SARS-CoV-2 commercial antibody assays are used for NSP work; the Abbott Architect SARS-CoV-2 IgG II Quantitative assay and the Roche Elecsys Anti-SARS-CoV-2 assay (anti-NP). Both assays are CE marked. Specimens are first screened using the Abbott SARS-CoV-2 IgG II Quantitative Assay which detects antibodies to SARS-CoV-2 spike protein (S) receptor binding domain. Specimens with a result of at

least 50.0 arbitrary units per millilitre (AU/mL) are considered positive (S+). Anti-spike positive specimens (S+) are subsequently tested using the Roche Elecsys Anti SARS-CoV-2 assay which qualitatively detects immunoglobulin G (IgG) antibodies to the SARS-CoV-2 nucleocapsid protein (N). Further information on these assays can be found in Appendix B

Interpretation of routine serosurveillance testing results:

Outcome	Interpretation
S-:	No antibodies to the spike protein detected
S+:	Antibodies detected to the spike protein (indicates prior infection and/or vaccination against SARS CoV 2
S+N-:	Serological results consistent with vaccination against SARS CoV only
S+N+:	Serological results consistent with prior infection with SARS CoV 2 (+/-vaccination for SARS CoV 2)

FACS testing

The Sample Eligibility criteria for inclusion in FACS testing are outlined as follows:

- Specimen sources: LSN adult hospitals and IBTS (adults aged ≥18 years). Excludes children aged 17 years or younger due to insufficient residual sera volume.
- Minimum sera volume: must meet minimum sera volume requirements, i.e. 500µl. Excludes specimens of insufficient volume, haemolysed, icteric or lipaemic.
- Target antibody: reactive on both the Abbott and Roche assays (S+N+), indicative of past infection (with or without vaccination) and S+N- (past vaccinationwith no serological evidence of infection). S- samples were excluded.
- Timeframe: specimens collected as part of routine NSP activities in January/February 2023 from both LSN network and IBTS

FACS Phase 1 minimum samples size calculations

Minimum sample size: A correlation coefficient measures the relationship between two variables: the Abbott Architect SARS-CoV-2 IgG II Quantitative assay result and the neutralising assay result. The recommended minimum sample size for a correlation *r* of 0.4 (for a simple correlation), a significance level (two-sided test) of 5%, and 80% power, stratifying for three age groups and two specimen sources (LSN and IBTS) is 282. Sample size options, based on varying correlation coefficient, are shown in Table 1. A correlation coefficient *r* of 0.4 is conservative and was recommended for this study.

Table 1 Minimum sample size calculations for Phase 1 of this study (testing using the cPass™ neutalisation assay)

	A	B	C	D	E	F
	Significance level (α)	**Power (1-β)**	**Correlation coefficient *(r)**	**n per age group**	**n all three age groups (D*3)**	**n total: LSN and IBTS (E*2)**
Antibody neutralisation (cPass™ assay)	0.05	0.2	0.4	47	141	**282**
	0.05	0.2	0.6	20	60	120
	0.05	0.2	0.7	13	39	78

*To detect a simple correlation *r* (r=0.4), the required sample size is 282. Note that *r* = 0.7 would be considered a high correlation coefficient and may be unrealistic, therefore 0.4 is recommended for this study. Sample sizes were calculated using Sample Size Estimation - Correlation (cuhk.edu.hk) Reference: Lachin (1981) Controlled Clinical Trials 2: 93-113

Sampling Strategy for FACS Phase 1

Specimens were stratified by Abbott Architect SARS-CoV-2 IgG II quantitative antibody level into four ranges (0 – 500 BAU/ml, 500 – 2500 BAU/ml, 2501 – 5679 BAU/ml) and then further stratified into three study age groups (18- 49 years, 50 – 64 years and 65+). Specimens from with these groups were randomly selected. For 65+ age groups, additional samples from the LSN were utilised to supplement for shortfalls in samples in this age group from IBTS dataset.

FACS Testing Phase 1: Testing using c-Pass neutralisation assay

The cPass™ neutralisation enzyme-linked immunosorbent assay (ELISA) from Genscript Biotech (CE marked) detects total neutralising antibodies (NAbs) without the need for any infectious virus or cells, and can be completed in one to two hours in a biosafety level two (BSL2) laboratory, enabling high throughput testing (16). Briefly, this assay is a blocking ELISA that takes advantage of the interaction of the receptor binding domain of the SARS CoV 2 RBD with hACE cell surface protein. The presence of functional antibodies in specimen neutralise or inhibit the interaction of RBD with hACE2 protein. High percentage neutralisation in this assay corresponds to high levels of functional antibodies. The readout is in the format of percentage neutalisation. The optional standards which allow for a semi-quantitiave readout will not be included in this study as they add little value, the arbitrary unit is not linked to an international standard at present; as such it would not be possible to interpret results in a meaningful way.

The test, which has been validated with two cohorts of patients with COVID-19 in two different countries, achieves 99.93% specificity and 95–100% sensitivity, and differentiates antibody responses to several human coronaviruses (16). GenScript

Biotech report that there is no cross reactivity with HCoV 229E, OC43, NL63 or HKU1. All cPass test kits used in phase 1 of this study were from the same lot number and expiry date (kits lot no: L00847-C with expiry date: 28/08/2023).

FACS Testing Phase 2: $TCID_{50}$ Neutralisation Assay testing using two SARS-CoV-2 viral isolates

The TCID50 test (infectious virus neutralisation test) aims to monitor cell destruction or cytopathic effect (CPE) of virus on an inoculated host cell culture. The ability of serum samples to inhibit viral infection, and therefore CPE is a reflection of neutralisation ability of sera.

Fully infectious virus samples were obtained from the European Virus Archive and The Centre for Emerging Pathogen Research (CEPHR). The clinical isolates were isolated from nasopharyngeal swabs collected from SARS-CoV-2 infected individuals. An isolate representing ancestral SARS-CoV-2 (2019-nCoV/Italy-INMI1) and the most recent SARS-CoV-2 variant available, omicron BA.5 from the All-Ireland Infectious Disease (AIID) cohort, were chosen for testing. The sample size for testing of sera was limited to approximately 20 due to the labour-intensive nature of the TCID50 assay. All laboratory procedures were performed in a biosafety level 3 (BSL3) laboratory in UCD.

SARS-CoV-2 (Ancestral): VeroE6 cells were plated on 96 well plates in DMEM/3% foetal bovine serum (FBS) with Penicillin/Streptomycin for 24 hours (100ul per well). Sample sera was diluted 1:2 with DMEM/3% FBS before mixing 1:1 with SARS-CoV-2 Ancestral (Itay-INMI-1) and incubating at 37°C for 30 minutes, before the mixture was titrated on VeroE6 cells. All virus used in this study was at passage 2. Cells were scored for cytotoxicity 72 hours post-inoculation, and only dilutions that demonstrated an absence of visible cytotoxic effect (i.e., rounded or detached cells) were scored in TCID50 assays following the method of Reed and Muench (1938). To differentiate between CPE and cytotoxicity following incubation with sera, each serum sample was titrated alone on VeroE6 cells at the same dilutions as used for TCID50 assays and evaluated for cytotoxicity.

SARS-CoV-2 (Omicron BA.5): VeroE6 cells were transfected with TMPRSS2 DNA to transiently express TMPRSS2, a protease utilised by SARS-CoV-2 variants (alongside ACE2) to gain cell entry ($VeroE6_{TMPRSS2}$). $VeroE6_{TMPRSS2}$ cells were plated on 96 well plates in DMEM/3% FBS with Penicillin/Streptomycin for 24 hours (100ul per well). Sample sera was diluted 1:2 with DMEM/3% FBS before mixing 1:1 with omicron BA.5 and incubating at 37°C for 30 minutes, before the mixture was titrated on $VeroE6_{TMPRSS2}$ cells. Cells were scored for cytotoxicity 72 hours post-inoculation, and only dilutions that demonstrated an absence of visible cytotoxic effect were scored in TCID50 assays. To differentiate between CPE and cytotoxicity following incubation with sera, each serum sample was titrated alone on $VeroE6_{TMPRSS2}$ cells at the same dilutions as used for TCID50 assays.

Controls for FACS Phase 2

A virus only sample not incubated with serum was used as a negative neutralisation control. An additional serum control was also included as an add on to testing. This serum sample was a commercially available pooled serum sample that has been treated to remove all antibodies (referred to as antibody negative sera). Antibody negative sera was tested by NVRL for anti-spike antibody and found to be negative. This serum sample was obtained from Sigma Alrdrich, product number H4522 and source was pooled male plasma donors from USA.

Analysis of Results from Phase 2 testing and Calculation of Neutralisation

Each serum sample for Phase 2 was tested twice in quadruplicate, which allowed two TCID50 results to be calculated per serum sample. The virus only/serum negative control with the highest TCID50 result was used as reference point in the calculation of percentage neutralisation. TCID50 and neutralisation are inversely related, therefore the percentage neutralisation was calculated as:
1 – (serum TCID50/no virus or serum negative TCID50)

The mean percentage neutralisation for each sample was calculated as the mean of the two TCID50 results per sample.

Study Ethical considerations

Serosurveillance is a core surveillance activity and residual blood samples are collected via the NSP under the 1981 infectious disease regulations and subsequent amendments. All samples collected are anonymised at source and contain a very limited dataset. This study was considered of public health importance for interpretation of serosurveillance data in the context of COVID-19 epidemiology and the ongoing implications of understanding immunity to COVID-19 in the Irish population.

Statistical Analysis

Statistical analysis was carried out in the statistic package, R & RStudio. Spearman's rank correlation coefficient was used to compare the datasets as it does not assume a linear trend in data. Segmented linear regression (also known as piecewise linear regression) was applied to investigate the association between the quantitative assay result and the neutralising assay result. The independent variable, quantitative assay result (measured in BAU/ml) was partitioned into intervals and a separate line segment was fit to each interval. The `segmented` package from R was used to estimate how many break points were required to fit the data and to estimate their location(s). The slopes (parameter estimates) for each line segment were obtained along with confidence intervals around the slopes.

Results

Overall Sample characteristics

Of 2,285 specimens collected during the Jan/Feb serosurveillance collection period, 287 specimens were selected as FACS samples for the C-pass neutralisation assay as they met the study inclusion criteria outlined in the methods (Diagram 1 & Table 2). FACS samples were randomly selected where possible with the main exception in the over 65 age group where sample numbers were small particularly in the IBTS cohort and all samples in a group were required to be selected to meet the minimum samples for that age group. It is not surprising that there are low numbers of blood donors captured in the over 65 year age group due to the criteria that have to be met to become a blood donor. Additional samples, therefore, in the over 65 age group were selected from LSN collection to supplement deficits in the over 65 age group from IBTS. A breakdown of the final FACs samples selected by age group, antibody level and source are shown in Table 3.

The FACS samples retained features of the overall Jan/Feb collection dataset (Table 4) including a high percentage of samples with hybrid immunity (78% in FACS samples compared to 85% in Jan/Feb collection) and high percentage of male to female samples (for example 57% male in FACS samples compared to 57% in Jan/Feb collection). The profile by age group in the FACS sample is not representative of the Jan/Feb collection as the study required specific number for specific age groups so that comparisons could be made. Characteristics of the FACS samples compared to the overall cycle collection samples from LSN and IBTS are shown in Table 4.

Diagram 1: Overview of testing pathway and sample sizes for FACS.

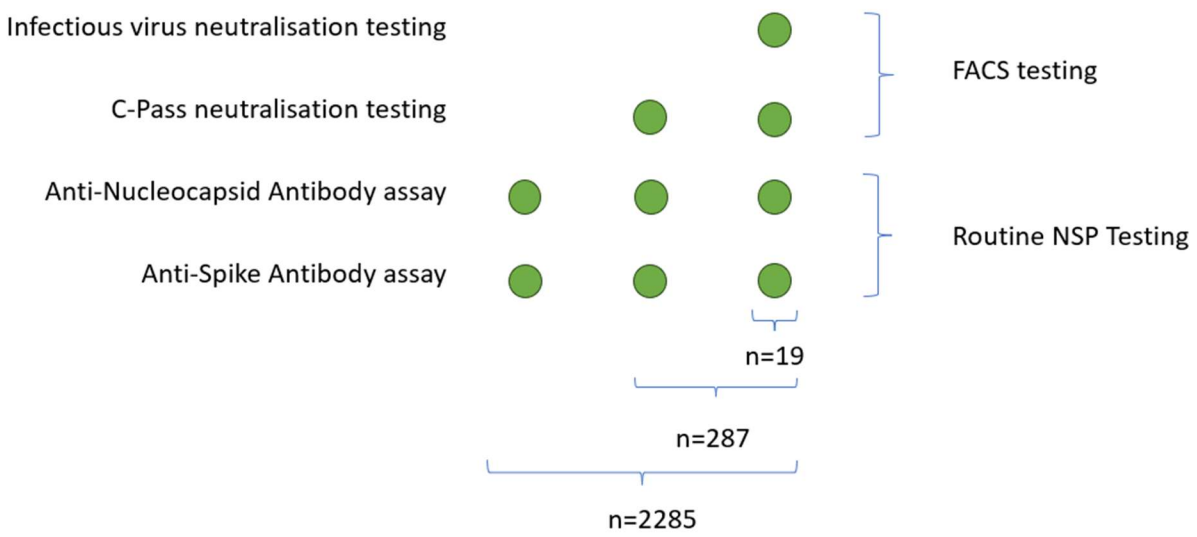


Table 2 – Jan/Feb 2023 serosurveillance collection timeframes from which FACS samples was drawn.

Collection Type	Dates of Collection	Total Samples Received	Number & percentage of Samples selected for Study
LSN Cycle 10	30/1/23 - 10/2/23	1223	162 (13%)
IBTS Week 3 & 5	16/1/23 - 21/1/23 & 30/1/23 - 4/1/23	1062	125 (12%)
Total	**Jan & Feb 2023**	**2285**	**287 (13%)**

Table 3 – Breakdown of FACS samples selected by age group, antibody level and source.

Quantitative Abbot Architect Antibody level (BAU/ml)	Source	18-49 years	50 – 64 years	65+ years	Total
<500	IBTS	12	12	1	25
<500	LSN	12	12	19	43
Total samples <500	Combined	24	24	20	68
500 – 2500	IBTS	12	12	10	34
500 – 2500	LSN	12	12	17	41
Total 500 – 2500	Combined	24	24	27	75
2501 – 5679	IBTS	12	12	10	34
2501 – 5679	LSN	12	12	14	38
Total 2501 – 5679	Combined	24	24	24	72
>=5680	IBTS	12	12	8	32
>=5680	LSN	12	12	16	40
Total >=5680	Combined	24	24	24	72
Overall Total	**Combined**	**96**	**96**	**95**	**287**

Table 4 – Characteristics samples collected during the of Jan/Feb serosurveillance collections and FACS samples.

	LSN				IBTS				All Specimens			
Characteristic	Number Cycle 10	Percent of Total S+	FACS Number LSN	FACS Percent of Total S+	IBTS Week 3 & 5	Percent of Total S+	FACS IBTS	FACS Percent of Total S+	Total Collection Jan/Feb*	Percent of Total S+	Total number FACS	FACS Percent of Total S+
S+ & S-	1223		162		1062		125		2285		287	
S+	1201		162		1055		125		2256		287	
S+N-	204	17%	46	28%	133	13%	16	13%	337	15%	62	22%
S+N+	997	83%	116	72%	922	87%	109	87%	1919	85%	225	78%
Female	594	49%	75	46%	382	36%	48	38%	976	43%	123	43%
Male	607	51%	87	54%	673	64%	77	62%	1280	57%	164	57%
18-49 yrs	704	59%	48	30%	644	61%	48	38%	1348	60%	96	33%
50-54 yrs	261	22%	48	30%	358	34%	48	38%	619	27%	96	33%
65+ yrs	236	20%	66	41%	53	5%	29	23%	289	13%	95	33%

*study collection timeframe

Phase 1: c PASS Neutralisation Results comparison with anti-Spike antibody levels (Abbot Architect assay)

Overall results

Comparison of quantitative anti-spike IgG levels with cPass neutralisation percentage inhibition demonstrated an initial linear response at low quantitative IgG levels with significant correlation (Spearman's rho = 0.6, $p<0.001$) which then plateaued with levels of neutralisation reaching above 95% (Fig 1).

The data was also plotted on a log scale in order to aid visualisation and interpretation of the data (Fig. 2).

While the majority of samples followed the trend identified, there were 3 samples with very high quantitative antibody levels that demonstrated very low neutralisation ability (<20%). Conversely, there were also 2 samples with very low quantitative antibody levels but extremely high (>95%) percentage neutralisation.

Stratification by Sex

Stratification of samples by sex demonstrated a similar trend to the overall dataset, with an initial linear response which plateaued rapidly (Appendix E). There was some divergence at low antibody concentrations by sex, however, this is not considered significant. For the majority of samples, the confidence intervals around the fitted lines for both males and females overlapped indicating that there was no significant difference in trend between males and females.

Stratification by Age Group

Stratification of samples by age groups demonstrated a similar trend to the overall dataset, with an initial linear response which plateaued rapidly for each of the three age groups investigated (Appendix E). For the majority of samples, the confidence intervals around the fitted lines for the 18-49 year olds, 50 – 54 year olds and 65+ year olds overlapped indicating that there was no significant difference in trend between age groups. Of note, 2 out of the 3 samples with very high quantitative antibody levels that demonstrated very low neutralisation ability (<20%) were in the 65+ age group.

Stratification by Sample Source

Stratification of samples by sample source demonstrated a similar trend to the overall dataset, with an initial linear response which plateaued rapidly (Appendix E). Some divergence of the IBTS data from LSN data is seen at low quantitative antibody levels however, this appears to be influenced heavily have 2 data points that have very high neutralisation at very low quantitative antibody levels. In addition the confidence

intervals around the IBTS data fitted line are wide. Therefore, the difference between the trend is not considered significant between IBTS and LSN samples.

Stratification by Immunity Status

Stratification of samples by immunity status (either hybrid immunity S+N+ or vaccination only, S+N-) demonstrated a similar trend to the overall dataset, with an initial linear response which plateaued rapidly (Appendix E). Further division of the data by immunity status and age group showed no significant difference in trend.

Regression modelling

Fitting a segmented linear regression model to the mean percentage neutralisation on c-Pass by the log of quantitative anti spike antibody (Fig. 3) estimated that one break-point at 417 BAU/ml provided the best fit, with a slope of 0.2341 (95% confidence intervals: 0.1898 – 0.2784) estimated for the first fitted line and slope of -0.003 (95% confidence intervals: -0.0245 – 0.0190) estimated for the slope of the second fitted line. In other words, under 417 BAU/ml, mean neutralisation increases by approximately 23% for every 1 log increase in BAU/ml. Once 417 BAU/ml is reached, there is no significant change in percentage neutralisation as anti-spike antibody BAU/ml increases. The breakpoint reflects the point at which assay saturation is reached.

Fig. 1: Plot of mean percentage neutralisation as determined by c-Pass assay against Abbott anti-spike IgG antibody concentration (BAU/ml). Analysis was performed in R.

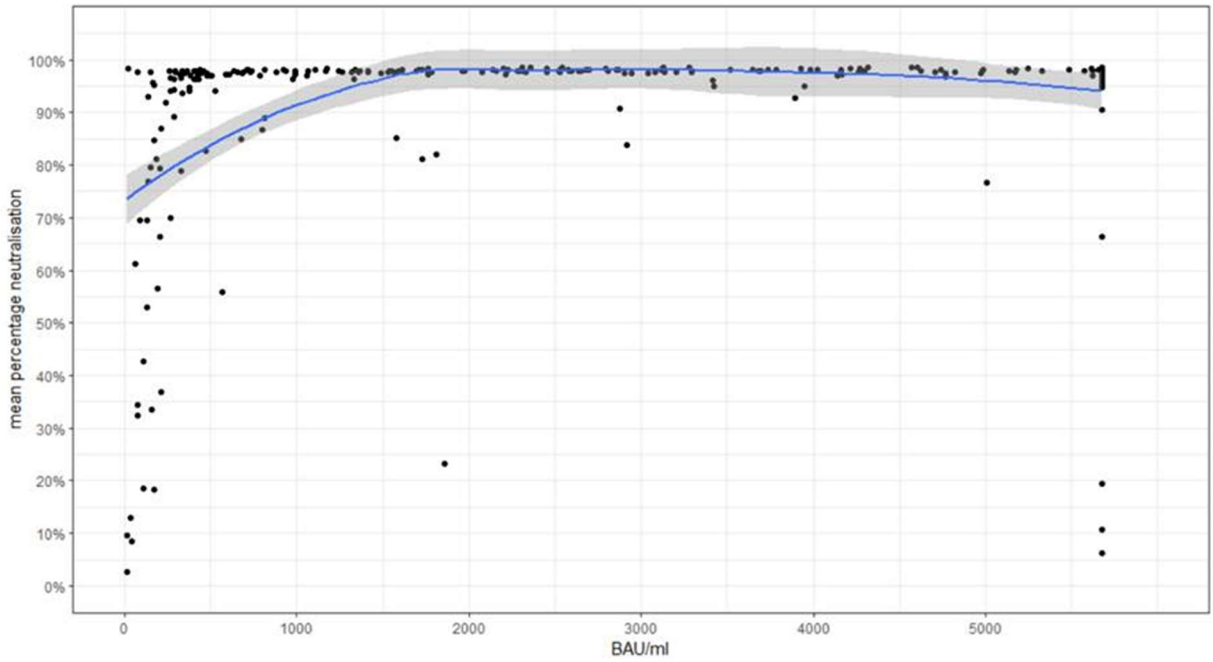


Fig 2: Plot of mean percentage neutralisation against log of anti-spike antibody concentration (BAU/ml). Analysis was performed in R.

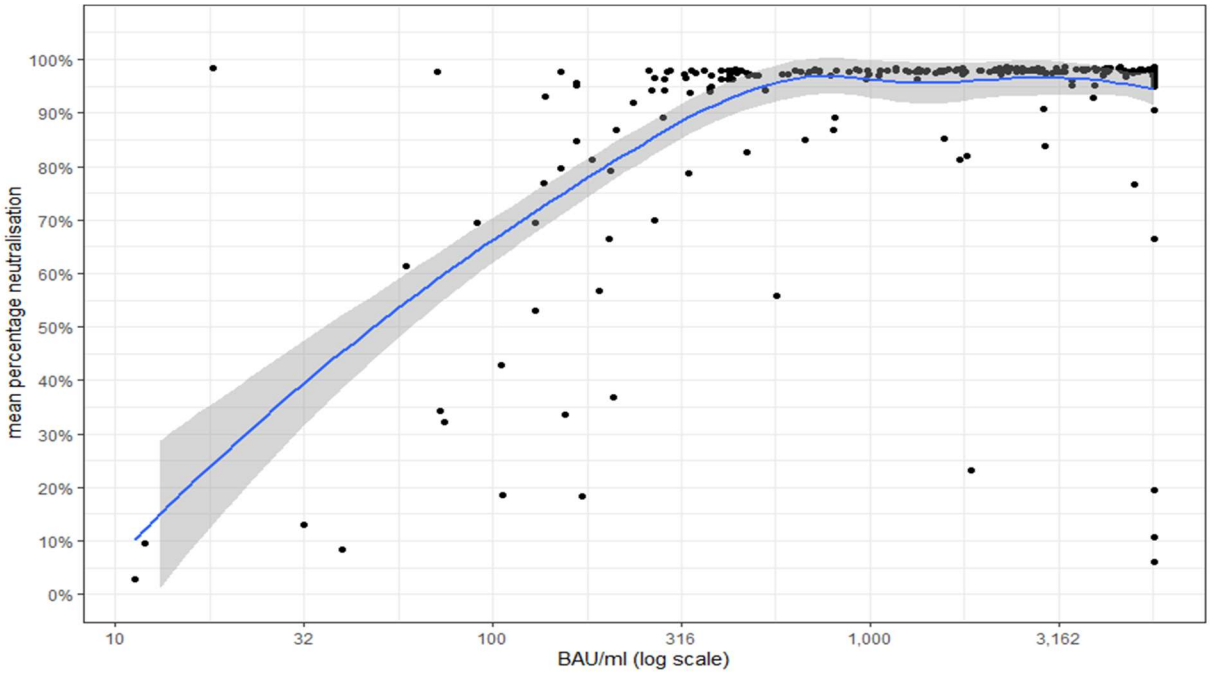
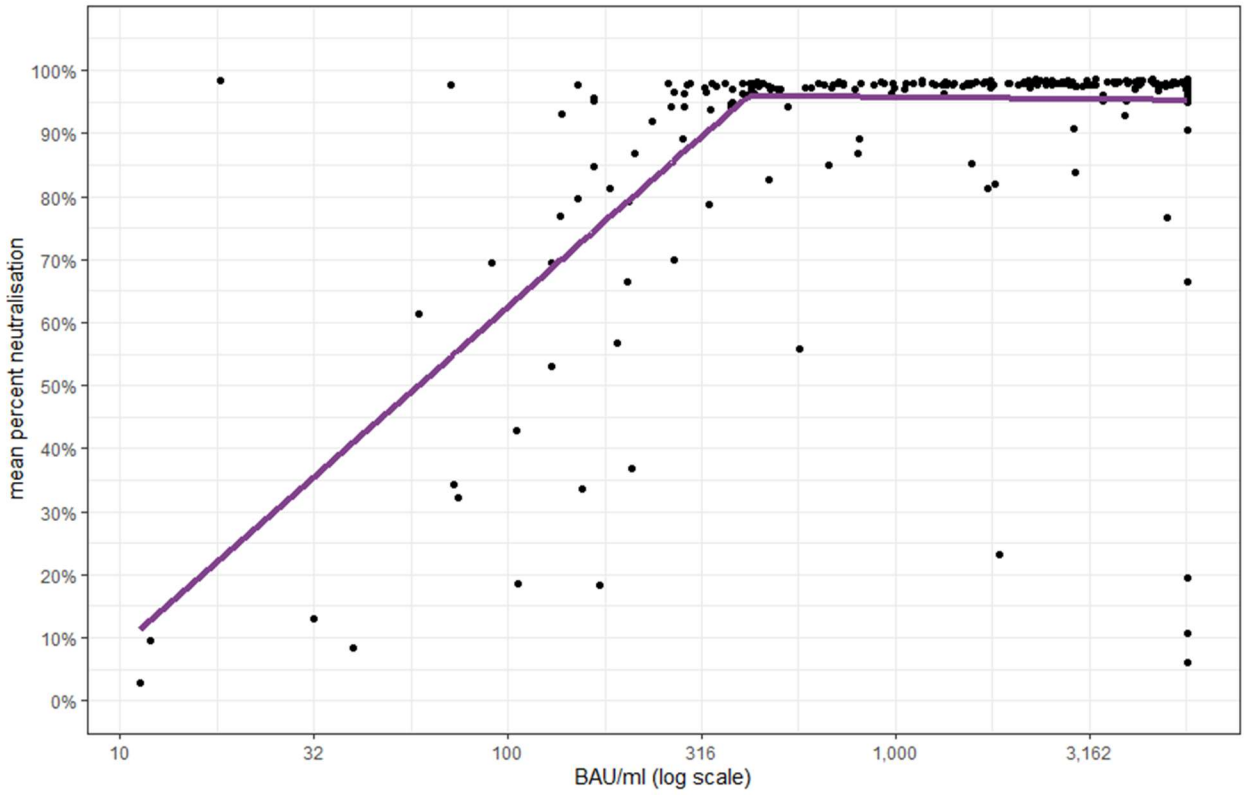


Fig. 3: Plot of Segmented Regression Model fitted to mean percentage neutralisation against log of anti-spike antibody concentration (BAU/ml). Statistical analysis was performed in R. The breakpoint between the two lines was calculated as occurring at 417 BAU/ml.



Phase 2: Serum neutralisation investigations using TCID50

In total, 19 serum samples were selected from Phase 1 to continue to Phase 2 testing to determine serum neutralisation ability against infectious SARS-CoV-2 (TCID50). These samples consisted of:

- 10 samples were randomly selected from the overall main trend seen in Phase 1 (serum samples T001 – T010)
- 1 sample was tested that was used as positive control across all C-Pass plates from Phase 1 and demonstrated high level neutralisation (serum sample T011)
- 8 remaining samples were chosen from the set of samples that did not fit the overall trend seen on Phase 1 testing (serum samples T012 – T019) as follows:
  - 5 samples with high BAU/ml results but low neutralisation on c-Pass testing,
  - 2 samples with low BAU/ml results but high neutralisation on c-Pass testing
  - 1 sample with mid-range BAU/ml result but low neutralisation on c-Pass testing

Testing also included a commercially available antibody negative serum sample and a virus only/no serum sample as negative controls. The characteristics of the 19 Phase one samples selected are shown in Table 5 and samples were well distributed across the range of characteristics. Overall results for Phase 2 are shown in Table 6.

Table 5: Characteristics of 19 serum samples chosen for testing in Phase 2.

Characteristic	Type	Number Specimens (Phase 2)
Age Group	18 – 49	8/19
	50 – 54	5/19
	65+	6/19
Sex	Male	10/19
	Female	9/19
Source	IBTS	10/19
	LSN	9/19
Antibody Group	<500 BAU/ml	10/19
	500 – 2500 BAU/ml	2/19
	2501 – 5679 BAU/ml	2/19
	>=5680 BAU/ml	5/19

Table 6: Overall Results of testing from Phase 2 by sample

Code	Description	ig_gii_quant_bau_ml	BAU/ml Range 4	Mean % Neutralisation C-Pass	Mean Neutralisation infectious virus assay Ancestral virus	Mean Neutralisation infectious virus assay omicron BA.5 virus
C001	Antibody negative serum sample	**Negative**			0%	86%
C002	No serum, virus only	**No Serum**			0%	0%
T001	Trend comparison	11.2	<500	3%	75%	28%
T002	Trend comparison	31.6	<500	13%	95%	100%
T003	Trend comparison	74.2	<500	32%	100%	100%
T004	Trend comparison	105.2	<500	43%	100%	85%
T005	Trend comparison	129.3	<500	69%	100%	100%
T006	Trend comparison	204.3	<500	79%	100%	77%
T007	Trend comparison	402.6	<500	98%	100%	100%
T008	Trend comparison	1765.1	500-2500	97%	100%	100%
T009	Trend comparison	4158.5	2501-5679	98%	100%	100%
T010	Trend comparison	5680.0	>=5680	97%	100%	100%
T011	Positive control used across c-Pass plates	284.3	<500	94%	100%	100%
T012	High BAU & Low neutralisation	5680.0	>=5680	19%	100%	100%
T013	High BAU & Low neutralisation	5680.0	>=5680	66%	100%	100%
T014	High BAU & Low neutralisation	5680.0	>=5680	11%	100%	100%
T015	High BAU & Low neutralisation	5680.0	>=5680	6%	100%	100%
T016	Very Low BAU & High neutralisation	18.2	<500	98%	100%	100%
T017	Very Low BAU & High neutralisation	71.0	<500	98%	100%	42%
T018	Mid-Range BAU & Low neutralisation	1859.1	500-2500	23%	100%	100%
T019	High BAU & Lower neutralisation than other samples	5007.9	2501-5679	77%	100%	100%

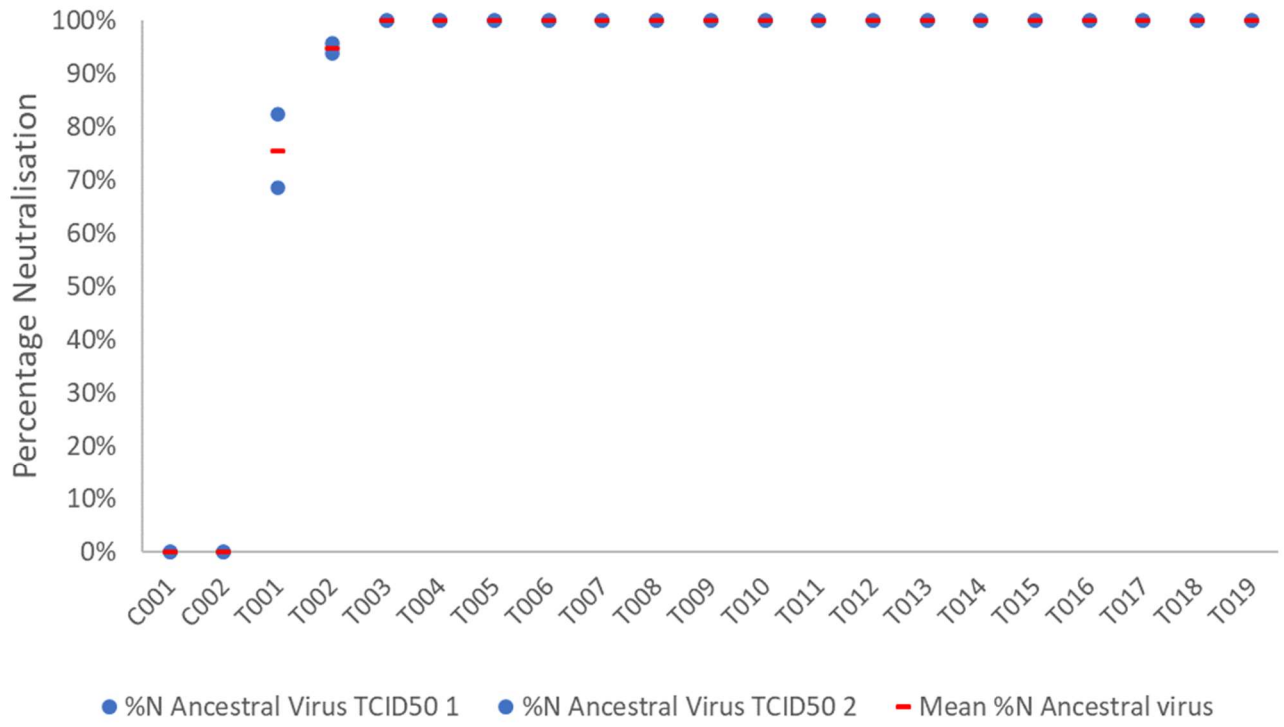
*4 replicates were run to calculate a TCID50 result. Two TCID50 results were available for each serum sample. A percentage neutralisation was calculated for each TCID50 result and the mean percentage neutralisation is presented in this table.

Ancestral SARS-CoV-2 Results

Seventeen out of the 19 selected serum samples from Phase 1 showed full neutralising ability/protective effect against Ancestral SARS-CoV-2 (Italy-INMI1) with mean neutralisation for these samples of 100% (Table 6 and Fig 6). Only two samples with very low BAU/ml showed lower neutralisation capacity but even this neutralisation result was over 60%.

Both virus only and antibody negative serum showed high level of virus infectivity of host cells and CPE translating as no neutralising ability or protective effect as expected.

Fig 6: Plot of neutralisation for serum samples selected for Phase 2 testing against Ancestral virus. Each serum sample was tested twice in quadruplicate to obtain a TCID 50 result which was converted to neutralisation. Mean Neutralisation was also calculated for each sample.



Omicron BA.5 variant Results

Fifteen out of the 19 serum samples from Phase 1 showed high levels of neutralising ability against omicron BA.5. Four serum samples showed reduced neutralising ability/protection against omicron BA.5 (Table 6 and Fig. 7 & Fig. 8).

The virus only control showed high level of virus infectivity of host cells and CPE translating as no neutralising ability or protective effect as expected. However, the antibody negative serum produced a low TCID50 result, i.e. low CPE in host cells which equated to high neutralising ability which was an unexpected result.

There are a number of possible explanations for this unexpected result including:

- This is a non-specific serum effect – the antibody negative sample is a pooled sample which can lead to some problems in assays. Therefore, this sample is different than other individual sera used in this study.
- There could be more cross reactivity between Omicron and other seasonal corona viruses. In other words, functional antibodies from other seasonal coronaviruses in this pooled sample may be cross reacting with Omicron and giving this unexpected result.
- Under the conditions of this round of testing, this serum sample inadvertently promoted cell growth which interfered with the result of testing. This serum is normally used in tissue-engineering, transplantation and cell therapy applications for the expansion of cell lines.
- There was some experimental error with this sample (although the replicates in this round of testing gave the same result)

Overall, it was considered that this result does not negate the results with other serum samples considering that the virus only/no serum control was showed 0% neutralisation as expected. This result was, therefore, excluded from further interpretation for this study.

Fig 7: Plot of neutralisation for serum samples selected for Phase 2 testing against omicron B.A.5 variant. Each serum sample was tested twice in quadruplicate to obtain a TCID 50 result which was converted to neutralisation. Mean neutralisation was also calculated for each sample.

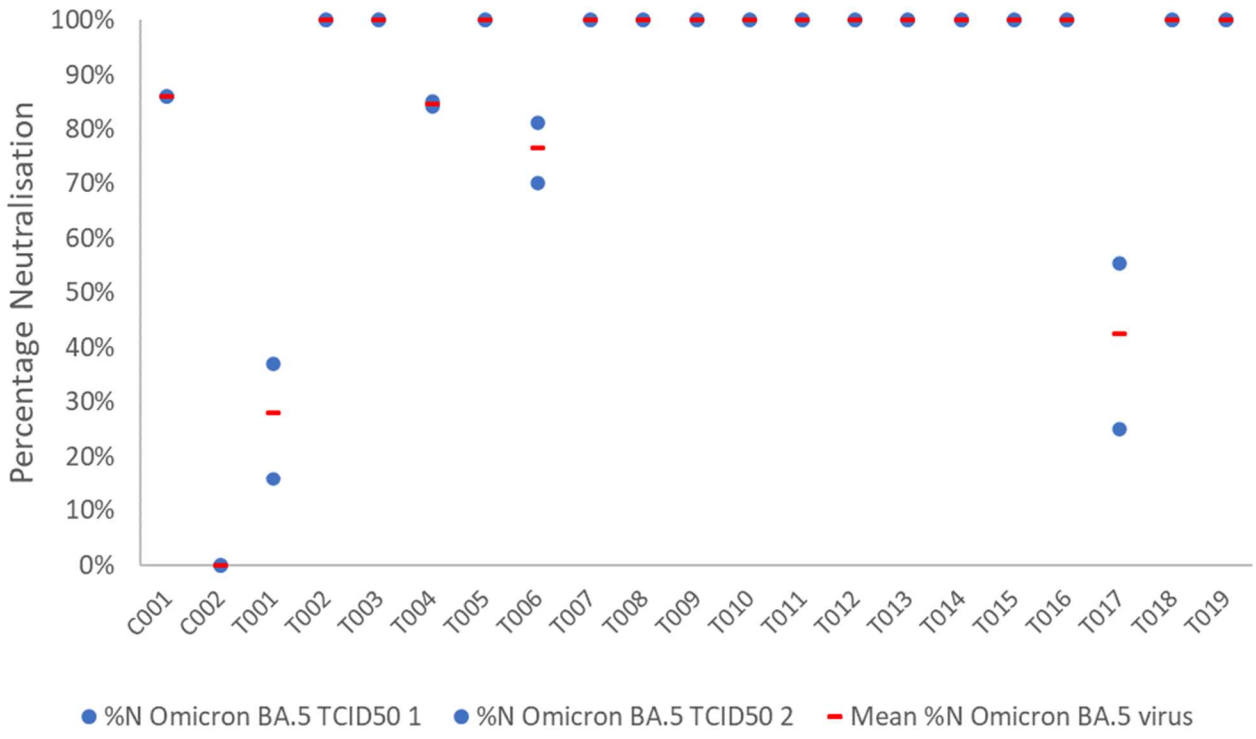
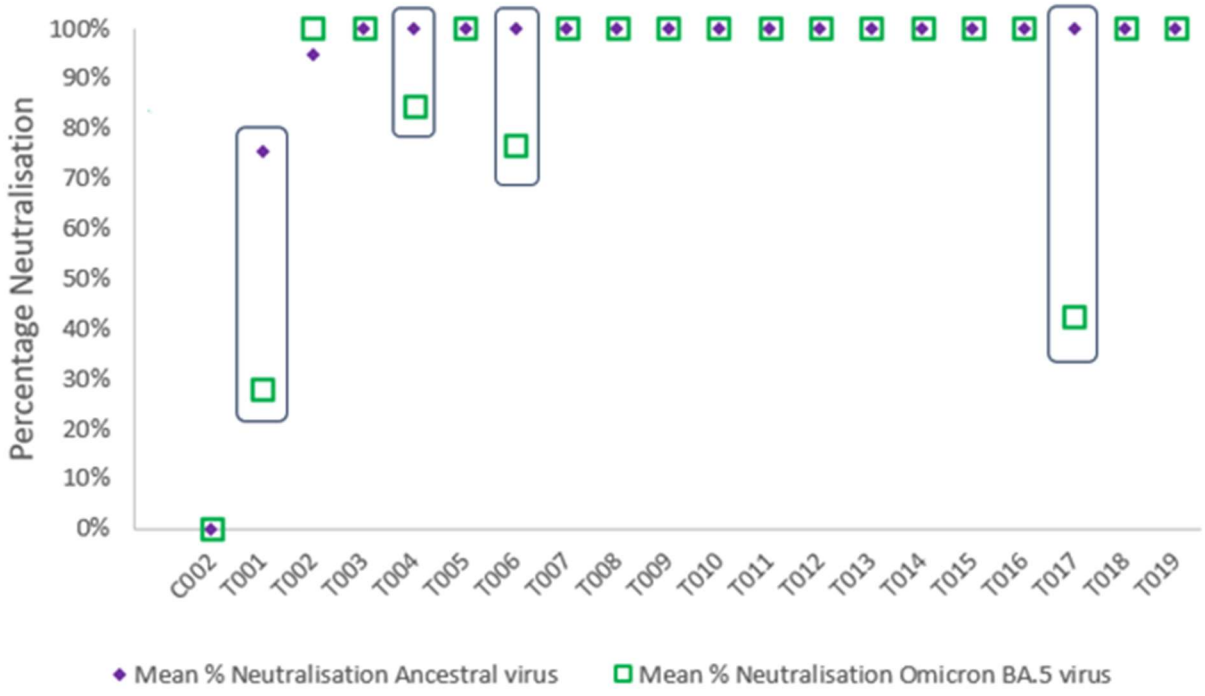


Fig 8: Plot of mean percentage neutralisation for serum samples selected for Phase 2 testing against ancestral SARS-CoV-2 and omicron B.A.5 variant. Each serum sample was tested twice in quadruplicate to obtain a TCID 50 result which was converted to neutralisation. Mean neutralisation was also calculated for each sample. Samples showing reduced neutralisation for omicron BA.5 compared to ancestral virus for the same serum sample are highlighted with black box in the plot to aid visualisation.



Discussion

This study demonstrated high levels of neutralising antibody in the population tested. The overall trend in neutralising ability observed using c-Pass assay was consistent regardless of age, sex, source of sample (healthy blood donor or primary care) or immunity status (likely vaccination only or hybrid immunity). The observation of neutralisation using the TCID50 assay protecting against SARS CoV 2 ancestral virus and the omicron BA.5 variant provides confirmation of the results detected using the c-Pass assay. These results are in line with results from other studies that demonstrate that anti-spike antibody quantitative levels correlate with neutralisation (e.g. Salazar et al., 2020; Valdivia et al., 2021; Moscato et., 2021; Dinc et al., 2022, Tsuchiya et al., 2023)

Ireland's COVID-19 vaccination programme has been extremely effective with uptake rates of 99.9% in the over 65's and 96.4% in 18+ age group for primary vaccination. Subsequent booster uptake rates have remained high in the over 65s (Appendix E) but reduced as time has progressed in over 65s and over 18s likely due to vaccine fatigue/hesitancy and perceived threat. However, in the over 65s the 3rd booster uptake rate, which included BA.4 and BA.5 components, still achieved almost 50% uptake rates in this age group. Both the vaccination program and circulation of the virus have likely contributed significantly to the high levels of functional antibodies reported in this study.

It is reassuring that specimens with medium to high levels of anti-spike IgG detected using the Abbott anti-spike assay from individuals over 65 years, a large vulnerable population, generally showed levels of neutralisation over 95%. These results show that antibody quality and effector function in over 65's, at least in terms of serum neutralisation is comparable to younger age groups. Our study did not detect any evidence of immunsenescence in the humoral antibody response to SARS-CoV-2.

This study has also demonstrated that samples with vaccination only profiles showed significant neutralisation ability, demonstrating that vaccination produces a humoral response likely to contribute to protection. While, not the direct focus of this study, it is important to compare measures of vaccine schedule delivery (e.g. uptake rate statistics) with other sources of information for a number of reasons including protecting against under-reporting of uptake rates and identification of potential waning of antibody protection. Increased population vulnerability associated with decreased vaccination uptake, delayed uptake due to vaccine hesitancy and new evolving variants make seroepidemiology studies a source of information for decision making.

The break point determined in the study at 417 BAU/ml estimates when the neutralisation assay is saturated, however, it is important not to infer that this equals protection. The real value of this study is showing that antibody quality and neutralising capacity relative to quantity does not vary between groups. The relationship between anti-spike antibody levels as determined using the Abbott anti-spike assay and neutralisation is important as it provides an opportunity to observe possible declining or changing neutralising antibody trends in the population. The ongoing monitoring of anti-

spike antibody levels using high throughput anti-spike commercial assays as a proxy for neutralising antibodies may provide valuable triangulation in decision making regarding the requirement for vaccination boosting or cohort boosting e.g. for immunocompromised. At an individual level, anecdotal evidence from Irish clinical settings suggests that break through infections have been seen for patients with anti-spike antibody levels of less than 900 BAU/ml, however this is not usually associated with severe illness. This would be in keeping with the results seen in this study.

It is interesting that four of the 19 sera (21%) selected for Phase 2 showed some reduced neutralisation ability against omicron BA.5. This variant was circulating between May 2022 and March 2023 (see Appendix D). Samples for this study were collected in Jan and Feb 2023. It is possible that this reduced neutralisation for some samples reflects lack of neutralising antibodies with high activity omicron BA.5. This may be due to a number of factors including lack of exposure to this specific variant, and possible variant immune escape characteristics. Interestingly, serum samples collected prior to the arrival of omicron variants have been shown to still confer neutralising ability against omicron variants albeit with some at reduced levels (Decru et al., 2022). Currently, parental lineages of BA.5 are no longer circulating in Ireland and XBB (originating from recombination of BA.2 descendants) and other recombinant SARS-CoV-2 variants have dominated (as of 1 November 2023, Appendix D). Unless there are further substantial mutational changes to the receptor binding domain of the spike protein which results in antigenic changes to the RBD a similar variable neutralisation pattern as observed in this study would be likely to be seen for newer variants.

In terms of clinical factors that may influence the relationship between anti-spike antibody and neutralisation, time since previous immune stimulation by vaccination and/or infection may also be an important aspect in terms of neutralising antibody levels. Declining antibody levels following vaccination is not unexpected and is frequently observed for a wide range of viruses such as hepatitis B and measles, however despite this decline clinical protection is maintained overall. Levin et al., while finding a significant relationship been quantitative IgG anti-spike antibody and neutralisation, found that this relationship was dependent on time since 2nd dose in vaccinated healthcare workers. Other studies have shown good neutralisation in vaccinated individuals (Grunau et., 2022; Xue et al., 2022). The humoral response in those previously infected with SARS-CoV-2 appears to be more durable with a number of studies indicating that neutralising antibody levels decrease only modestly after infection (Dan et al., 2021; Vanshylla et al., 2021). Symptom severity has also been studied as a factor which may influence variability in antibody titres (Chansaenroj et al., 2022). It was not possible to collect such clinical information for this study sample due to the legal framework governing sero-epidemiological surveillance but further research in these areas would be useful to increase our understanding of protective immunity.

Technically, it is not feasible to do infectious virus neutralisation studies on large numbers of specimens due to a number of factors, including the requirement for biosafety level three measures when handling infectious virus and the time and expense involved in infectious cell culture. Publication of this study will provide information should a systemic review or a meta analysis be undertaken in the future.

This work adds to the evidence base regarding the assessment of humoral protection against SARS-CoV-2 in an Irish context and will inform ongoing evaluation of vaccination strategies and other public health interventions. It is reassuring that the quality of antibody response is similar across age groups studied, and that neutralisation capacity relative to antibody quantity does not vary with likely vaccine induced immunity, relative to hybrid immunity. Hence, this work provides an evidence base that underpins the use of commercial, quantitative, readily available assays in the serosurveillance programme in Ireland as essential tools in the understanding of population immunity.

Limitations

- A residual serum sampling strategy involving anonymised specimens, by its nature, allows for only a minimal dataset to be collected with each specimen. No information will be collected on clinical history or Major Histocompatibility Complex (MHC) group, symptoms of SARS-CoV-2, vaccination status, ethnicity, country of birth or deprivation level.
- Paediatric samples were not included in this study. Furthermore, other population groups may not be represented, such as patients who are severely ill or immunocompromised, as specimens sent to the LSN originate from GPs and samples sourced from the IBTS originate from blood donors (i.e. the healthy population).
- Samples from IBTS and LSN were prepared in different manners i.e. IBTS samples were whole blood samples separated by gel (ficoll) whereas LSN samples were serum samples. Gel interference for antibody assays is uncommon. Furthermore, the C-Pass assay is robust for plasma or serum. From the results, no significant difference in trend was observable and high neutralisation was observed for IBTS samples so it is unlikely that the preparation of the samples had any major impacted on result of study.
- It should be noted that not all NAbs are necessarily RBD-binding antibodies: prior studies with SARS-CoV show that antibodies to other regions in the S1 or S2 protein can also play a role in virus neutralisation. However, studies indicate that non-RBD-targeting antibodies, which can be measured in pVNT, but not in the cPass™ assay, are unlikely to play a major role in SARS-CoV-2 neutralisation (16).
- Although the cPass™ assay demonstrates a high level of specificity, some level of cross reactivity with Severe Acute Respiratory Syndrome Type 1 Coronavirus (SARS-CoV-1) sera has been previously shown, which is not unexpected given its close genetic relatedness with SARS-CoV-2 (16). Studies show presence of long-lasting NAbs to SARS-CoV-1 17 years after the initial infection. There was no cross reactivity demonstrated with Middle East respiratory syndrome (MERS-CoV) GenScript Biotech reported that there is no cross reactivity with HCoV 229E, OC43, NL63 or HKU1, but it would not be possible to verify this in our laboratories as we do not have assays to detect seasonal coronaviruses.
- The testing reported in this study is based on serum neutralisation, rather than neutralisation on the muscosal surface.

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Thank you to the partners who contributed to this work including:

1. The Laboratory Serosurveillance Network: Letterkenny University Hospital, University Hospital Limerick, Galway University Hospital, Tallaght University Hospital, Beaumont Hospital, St Vincent's University Hospital
2. Irish Blood Transfusion Service (IBTS)
3. UCD National Virus Reference Laboratory Serosurveillance Unit
4. UCD School of Veterinary Medicine, Dublin
5. Health Protection Surveillance Centre, Seroepidemiology Unit

Further information

This work is under the governance of the National Serosurveillance Programme (NSP) Steering Committee

For further information on the National Serosurveillance Programme see here

For further information on the Health Protection Surveillance Centre see here

To view the Seroepidemiology of COVID-19 data hub, please visit https://seroepi-hpscireland.hub.arcgis.com/

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Appendix A: Study Group Members

The FACS Study group members comprised healthcare professionals representing various professional groups and relevant agencies including the Health Protection Surveillance Centre's (HPSC) Seroepidemiology Unit (SEU), UCD National Virus Reference Laboratory's (NVRL) Serosurveillance Unit (SSU), UCD School of Veterinary Medicine, Beaumont Hospital Dublin, Irish Blood Transfusion Service (IBTS), Academy of Clinical Science and Laboratory Medicine, St James Hospital Dublin. The study group, which was chaired by Dr. Derval Igoe (Nov 2022 – Jan 2023) and Dr. Elaine Brabazon (Feb 2023 – Feb 2024), reported to the Steering Committee of the National Seroprevalence Programme. Further details regarding the study or study group can be obtained by emailing the HPSC SEU: seu.programme@hpsc.ie

Appendix B: Interpretation of Assay Results

Interpretation of results for the Abbott Architect SARS-CoV-2 IgG II Quant assay

Antibody range and interpretation of results for the Abbott Architect SARS-CoV-2 IgG II Quant assay is shown in Table A. Results are received by the HPSC SEU as AU/ml (0.0 AU/ml – 40,000 AU/ml). In order to facilitate comparison with international literature, the results (AU/ml) are converted into the WHO International Standard concentrations for anti-SARS-CoV-2 immunoglobulin, which is measured in binding antibody units per millilitre (BAU/ml), using the manufacturer's suggested conversion of AU/ml x 0.142.

Table A Interpretation of results for the Abbott Architect SARS-CoV-2 IgG II Quant assay.

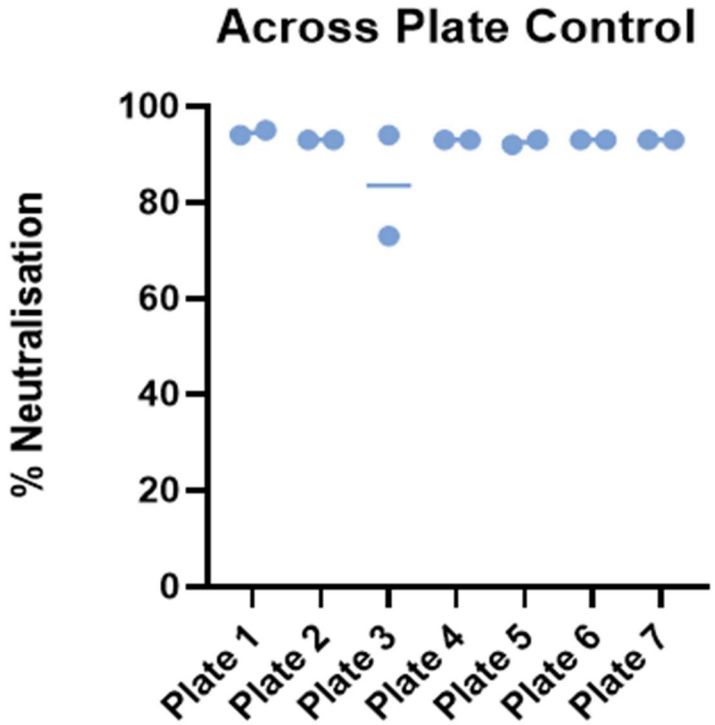
Unit	**Limits of quantification**		**Interpretation**	
	Lower	Upper	Negative	Positive
AU/ml*	21.0	40,000	< 50.0	≥ 50.0
BAU/ml	3.0	5,680	< 7.1	≥ 7.1

Appendix C: Inter and Intra Assay CVs

Testing was carried out in duplicate across seven assay plates. One sample was used as an internal control across all plates in addition to the assay negative control provided with the assay. The across plate control was replicated consistently apart from one reading which gave a significantly lower value (Fig A). The inter-assay coefficient of variation (average standard deviation/mean of duplicate values) was calculated as 3.98% when utilising all the across plate control and 0.76% when removing the lowest value from Plate 3. The most likely explanation for the low control result on Plate 3 is pipetting error. This cell specific issue is also supported by the fact that there was no unusual pattern in the overall results from samples from Plate 3 and samples run on Plate 3 followed the trend in the overall dataset (Fig. B).

The Intra Assay coefficient of variation was also calculated for all sample results. The average percentage CV for all samples was calculated as 1.6%. CVs below 10% for both inter and intra assay results are considered to have good reproducibility and consistency.

Fig. A: Neutralisation results for serum sample used as an across plate control for c-Pass assay. Duplicate results for this serum sample across the seven plates are shown.



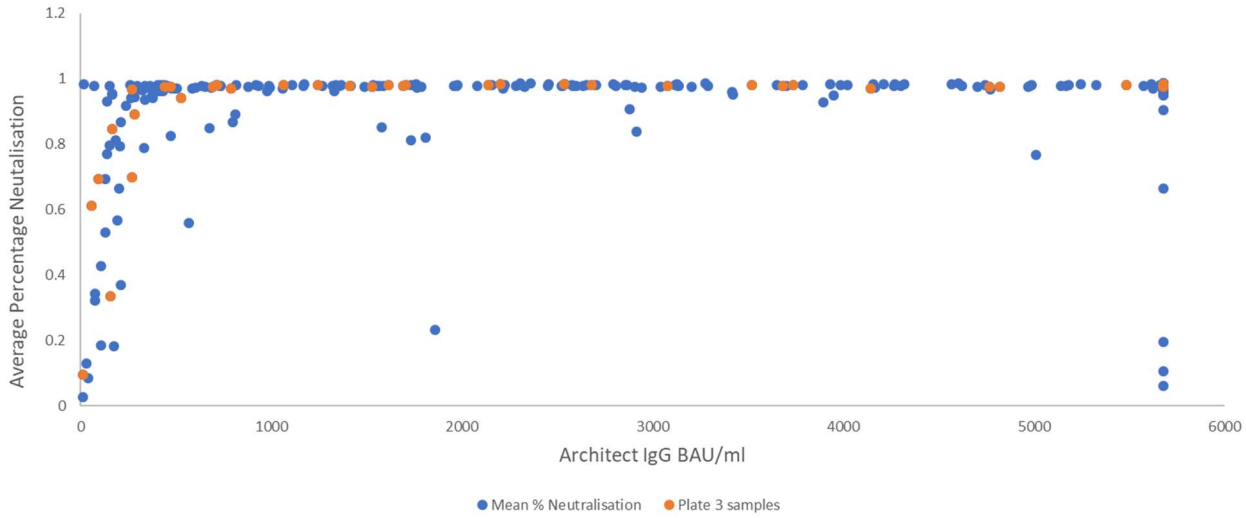
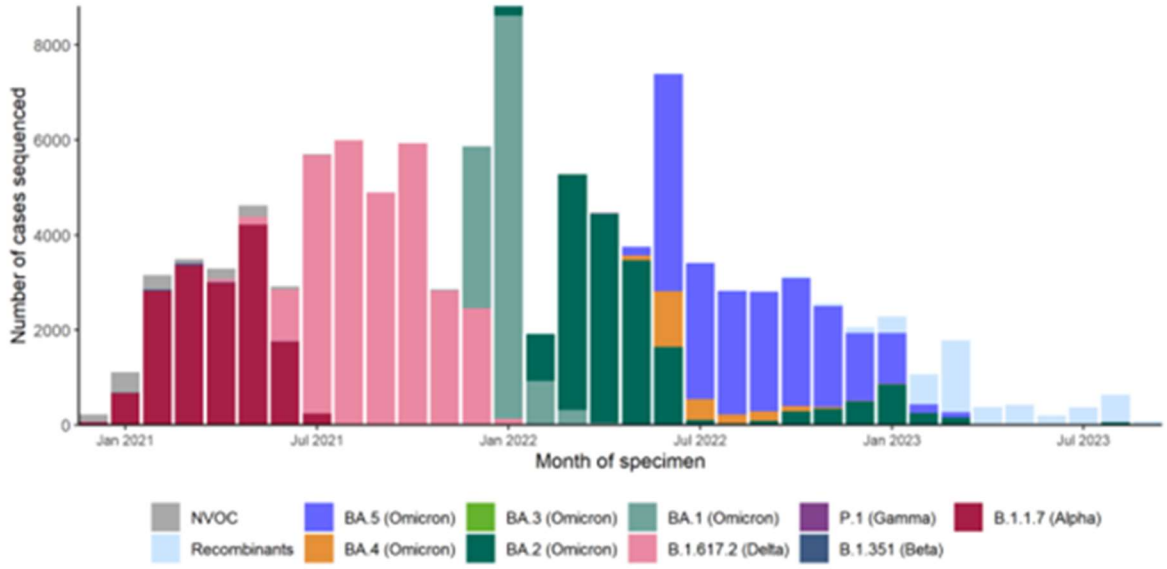


Fig. B. Plot of percentage neutralisation results for Plate 3 samples (orange) compared to all other samples. Plate 3 samples show a similar pattern to other samples without any clustering of results.

Appendix D: SARS-CoV-2 virus variant timelines in Ireland

The distribution of SARS-CoV-2 variants over time are shown in in Fig. C.

Fig. C: SARS-CoV-2 whole genome sequencing results (Ireland), specimen collection datas from December 2020 to September 2023. Source: Summary of SARS-CoV-2 virus variants in Ireland. Prepared by HPSC on 20/09/2023. Available at: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/surveillance/summaryofcovid-19virusvariantsinireland/Virus%20Variant%20report.pdf



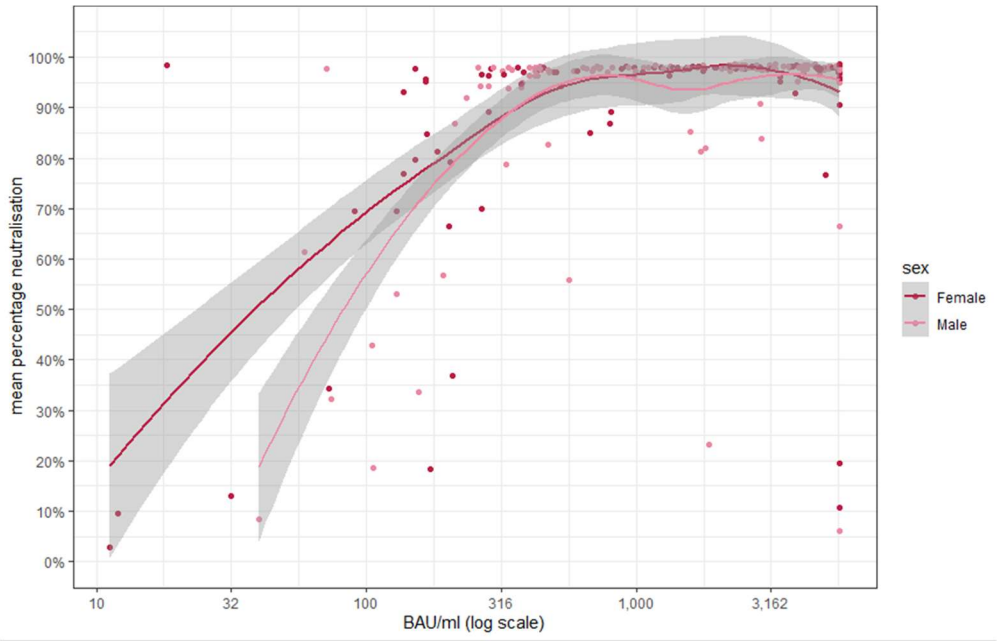
Appendix E: COVID-19 Vaccination timelines in Ireland for Adults

Timeline	Programme	Variants covered in vaccines	Uptake as of Week 16 2023 (wk ending 23rd April 2023)
Dec 2020	Primary Vaccination Programme for Adults	Original virus	65+ years: 99.9% 18+ years: 96.4%
Nov 2021	1st Booster for Adults (week 44, 2021)	Original virus	65+ years: 97.9% 18+ years: 81.1%
Apr 2022	2nd Booster for Adults (Week 14 2022) for specified cohorts	Original virus and the omicron variant BA.1	65+ years: 80.7% 18+ years: 34.4%
Oct 2022	3rd Booster for Adults (Week 39, 2022) for specified cohorts	Original virus and the omicron variant BA.4 and BA.5	65+ years: 49.7% 18+ years: 11.5%

Vaccination uptake reports are available at: https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/vaccination/covid-19vaccinationuptakereports/

Appendix F: C-Pass Neutralisation results comparison with anti-Spike antibody levels stratified by different variables

Plot of percentage neutralisation by anti-spike antibody level (log scale) by sex



Plot of percentage neutralisation by anti-spike antibody level (log scale) by age group

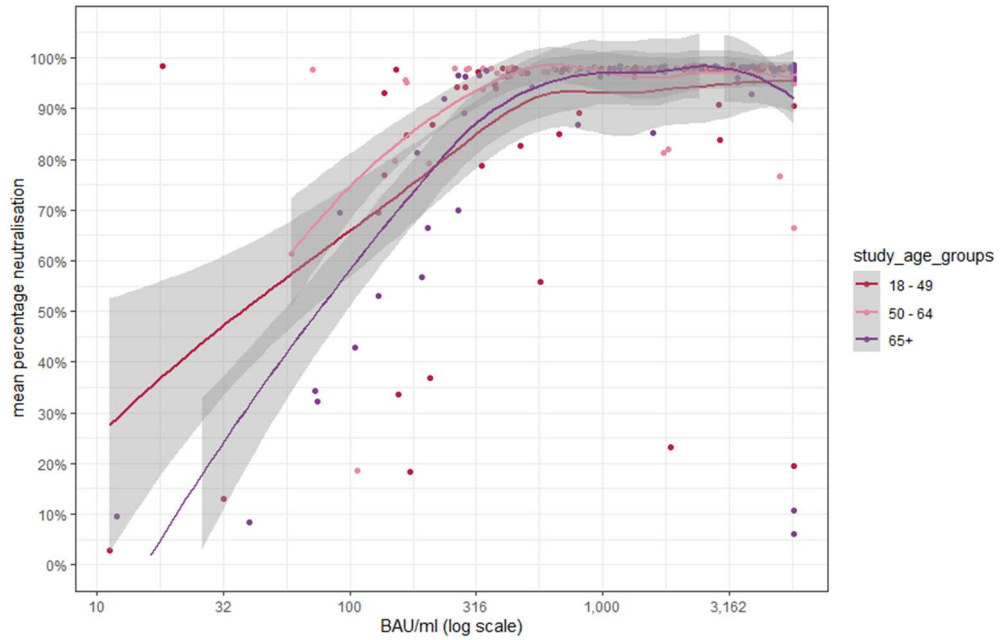


Fig. Plot of percentage neutralisation by anti-spike antibody level (log scale) by source

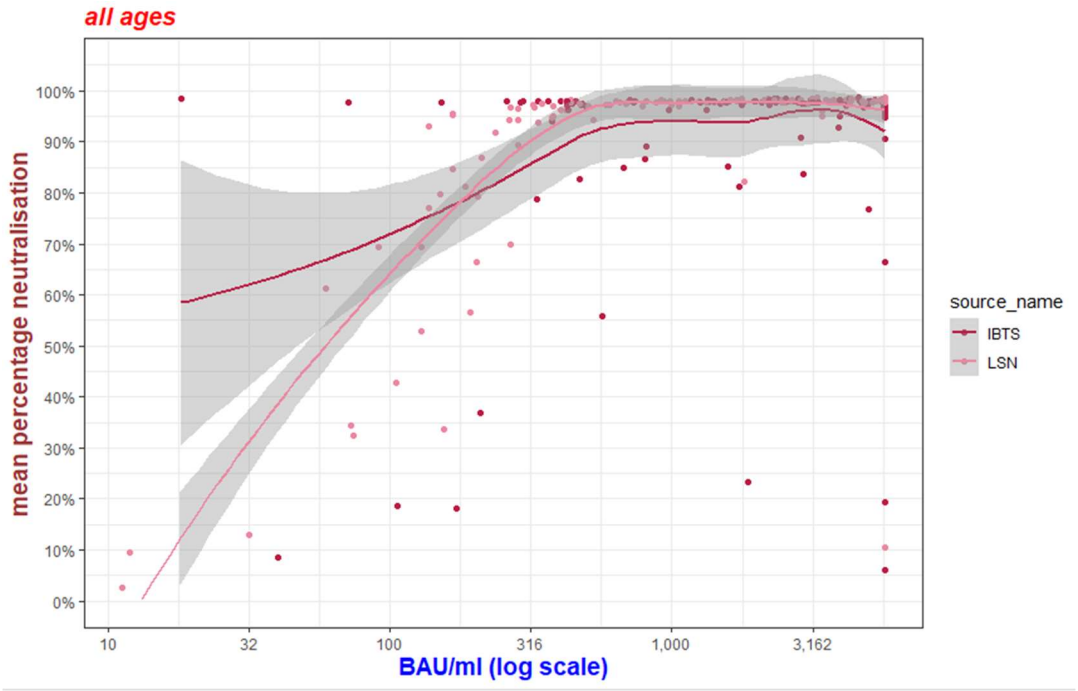
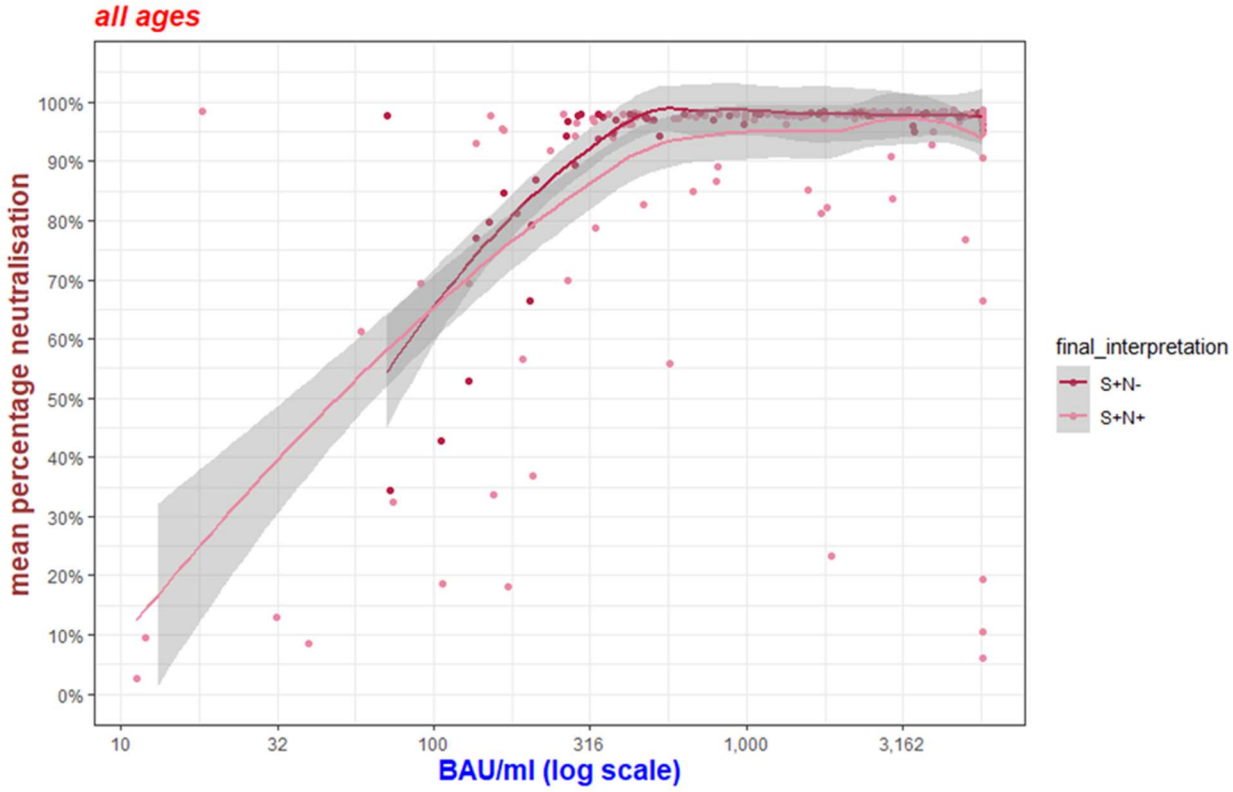
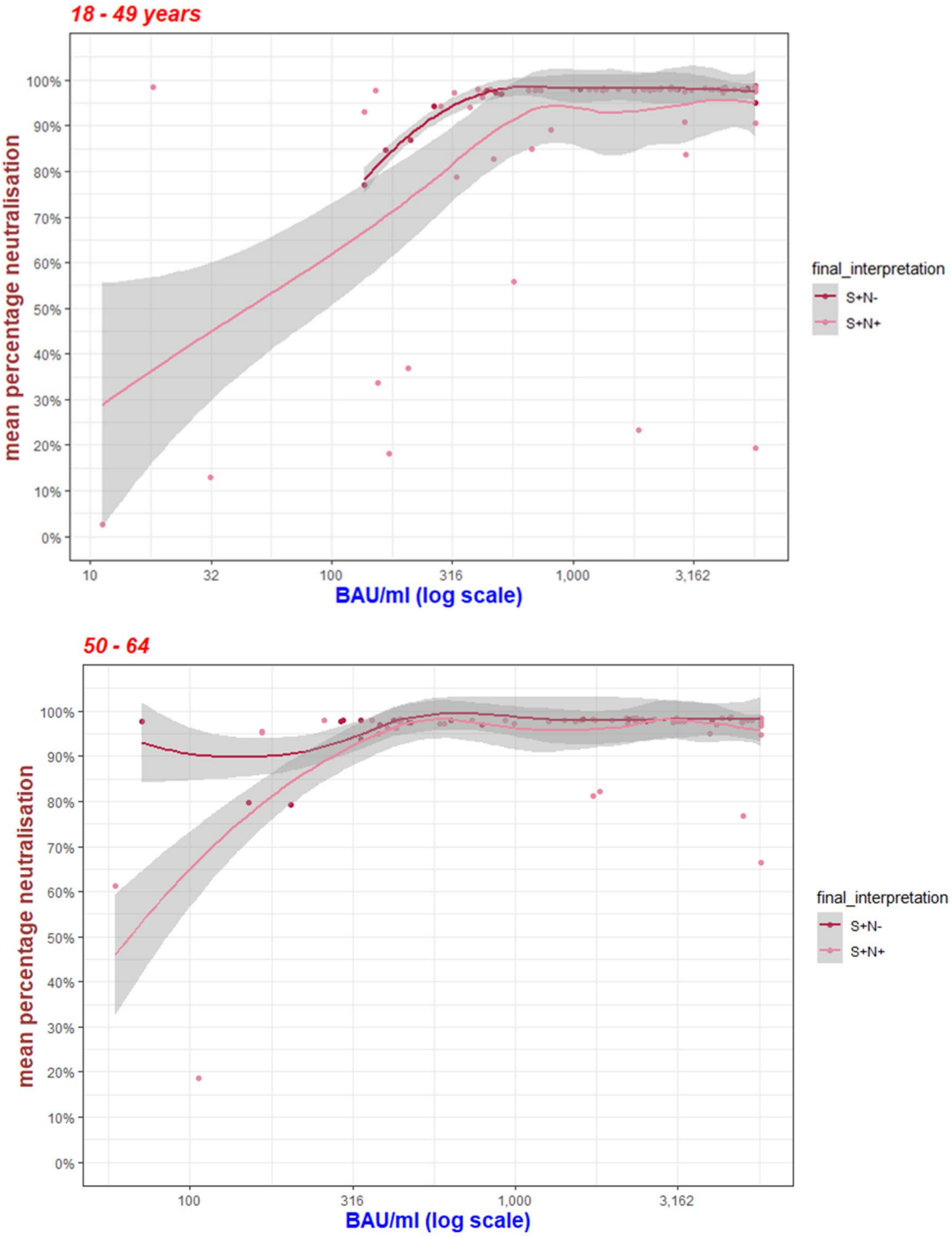
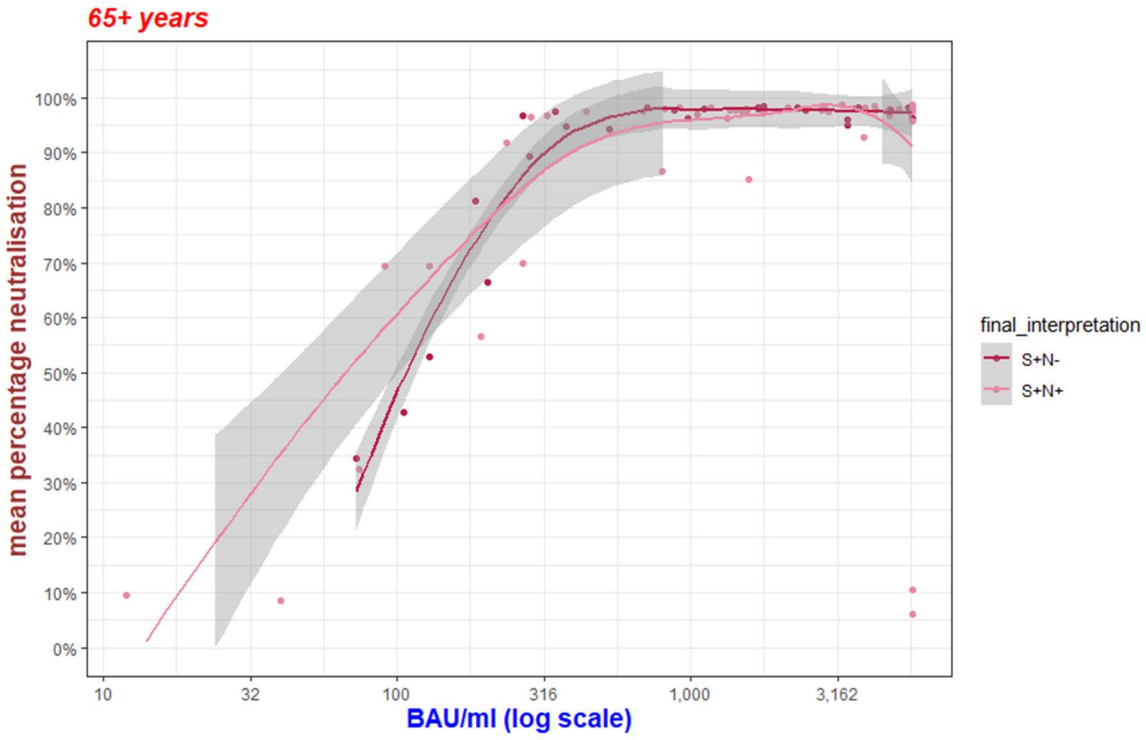


Fig. Plot of percentage neutralisation by anti-spike antibody level (log scale) by immunity status (all ages)



Plots of percentage neutralisation by anti-spike antibody level (log scale) by Immunity status by age groups




65+ years
mean percentage neutralisation
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
10
32
100
316
1,000
3,162
BAU/ml (log scale)
final_interpretation
S+N-
S+N+