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Saliva antiviral antibody levels are detectable but correlate poorly with serum antibody levels following SARS-CoV-2 infection and/or vaccination

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SUMMARY

The importance of salivary SARS-CoV-2 antibodies, following infection and vaccination, has not been fully established. 875 healthcare workers were sampled during the first wave in 2020 and 66 longitudinally in response to Pfizer BioNTech 162b2 vaccination. We measured SARS-CoV-2 total IgGAM and individual IgG, IgA and IgM antibodies. IgGAM seroprevalence was 39.9%; however, only 34.1% of seropositive individuals also had salivary antibodies. Infection generated serum IgG antibodies in 51.4% and IgA antibodies in 34.1% of individuals. In contrast, the salivary antibody responses were dominated by IgA (30.9% and 12% generating IgA and IgG antibodies, respectively). Post 2nd vaccination dose, in serum, 100% of infection naïve individuals had IgG and 82.8% had IgA responses; in saliva, 65.5% exhibited IgG and 55.2% IgA antibodies. Prior infection enhanced the vaccine antibody response in serum but no such difference was observed in saliva. Strong neutralisation responses were seen for serum 6 months post 2nd-vaccination dose (median 87.1%) compared to low neutralisation responses in saliva (median 1%). Intramuscular vaccination induces significant serum antibodies and to a lesser extent, salivary antibodies; however, salivary antibodies are typically non-neutralising. This study provides further evidence for the need of mucosal vaccines to elicit nasopharyngeal/oral protection. Although saliva is an attractive non-invasive sero-surveillance tool, due to distinct differences between systemic and oral antibody responses, it cannot be used as a proxy for serum antibody measurement.

Introduction

Serum antibodies to SARS-CoV-2, induced following infection and vaccination, have been well characterised in healthy and diseased cohorts[1–4] and are associated with protection from severe disease against all variants of concern to date.[5,6] In contrast, the role of salivary antibodies has not been full established.[7,8] Previous studies determined the presence of salivary IgG following natural infection[9–11] and vaccination[9,12,13] demonstrating persistent salivary IgG positivity following infection, high saliva positivity and incremental increases in salivary IgG concentrations following progressive vaccine doses. However, there are no large studies comparing serum and saliva to characterise the IgG, IgA and IgM antibody responses, following infection and vaccination.

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Mucosal immunity is likely to play an important role in protection against SARS-CoV-2, given that it is predominantly a respiratory acquired infection. SARS-CoV-2 is detectable in saliva and there is evidence that suggests salivary viral load correlates with disease severity and mortality.[14] Nevertheless, epidemiological evidence from the omicron wave suggests that vaccination does not prevent infection, despite a high proportion of the population having serum neutralising antibodies achieved either through prior infection or vaccination, but it does protect against severe disease. Having a deeper understanding of the humoral immune system present within the mucosal compartment of the body may provide an evidence base for this phenomenon and potentially inform future vaccine development strategies.

We have examined anti-spike glycoprotein (Spike) responses in paired serum and saliva obtained from a large health care worker cohort following natural infection and vaccination with Pfizer0 BioNTech COVID-19 vaccine (BNT162B2) (PFZ) vaccine and examined the capacity of a subset of representative samples to neutralise SARS-CoV-2 infection in vitro. Our group has previously developed and validated sensitive and specific assays that can detect SARS-CoV-2 specific antibodies of all immunoglobulin classes in saliva.[15]

Materials and methods

Ethics and patient samples

Paired serum and saliva samples were collected from healthcare workers (HCWs) at University Hospitals Birmingham NHS Foundation Trust as part of the COVID-19 Convalescent immunity (CoCo) study.[2] Baseline serum and salivary samples from 875 HCWs were collected between 27/04/2020 and 08/06/2020 during the first wave of the SARS-CoV-2 pandemic and post-vaccination samples were collected between 29/03/2021 and 24/12/2021 (Table 1). The study was approved by the London – Camden & Kings Cross Research Ethics Committee reference 20/HRA/1817. For 217 participants, who had detectable salivary antibodies, individual IgG, IgA and IgM isotypes were quantified in saliva and serum to better characterise this antibody response.

A more limited set of patients were then followed longitudinally, and we present serum and salivary data on 66 HCWs who subsequently received 2 doses of PFZ and 20 HCWs who received a third dose of PFZ. There were no significant differences between the baseline and longitudinal post-vaccination follow-up cohort (Table 1). Due to the well-documented effect of vaccination post-natural infection on antibody levels,[16] the results were analysed according to the presence or absence of anti-Spike antibody levels in the baseline serum sample. In the first wave of the pandemic, access to PCR testing was limited. Therefore, seropositivity was deemed a sensitive biomarker for past infection.[15] Participants were sampled 28–51 days post-2nd dose and 28–55 days post-3rd dose of vaccination. Forty-nine paired samples were available with sufficient volumes to perform neutralisation assays. These studies were undertaken on samples taken 6 months after their second vaccination (V2 +6 months) and 28–55 days post-3rd dose of vaccination.

Table 1
Demographic details for the whole cohort at baseline and sub-study of vaccinated participants.

	All Participants (Baseline)	Vaccine Participants
Total cohort size	N = 875	N = 66
Age (Median (IQR)	41 (31.0–50.0)	43 (31.8–51.3)
Gender, N (%)		
Male	236 (26.9%)	16 (24.2%)
Female	621 (71%)	50 (75.8%)
Not Stated	18 (2.1%)	0 (0%)
Ethnicity, N (%)		
White	636 (72.6%)	49 (74.2%)
Mixed	20 (2.3%)	3 (4.7%)
Asian	152 (17.4%)	11 (16.7%)
Black	32 (3.7%)	0 (0%)
Other	24 (2.7%)	3 (4.55%)
Not Stated	11 (1.3%)	0 (0%)

Median and Interquartile Ranges (IQRs) are provided for Age. There were no significant differences between the baseline and vaccine participant groups. Age was compared using a two-tailed unpaired Mann- Whitney test (P = 0.2). Categorical data were compared using the $\chi 2$ test (Gender - $\chi 2$ (2, N = 941) = 1.726, P = 0.4 and ethnicity $\chi 2$ (5, N = 941) = 5.3, P = 0.4.

Serum samples were obtained from whole blood after centrifugation at 1643*g* for 5 min and were stored at −20 °C until assay. Whole saliva samples were collected by drooling into 50 ml saliva collection tubes for a timed period of 4 min[17] All saliva samples were stored and transported on ice. Samples were centrifuged (2147 *g* for 10 min) to separate cells and insoluble matter, and the supernatant removed and frozen at −20° within 4 h. On the day of assay, samples were thawed and micro-centrifuged (10,621*g* for 10 min) prior to experiments. Saliva secretion can be influenced by numerous factors and to standardise this, all patients completed a 4-minute timed passive drool where they refrained from eating, smoking and brushing teeth in the 30 min prior to sampling and we assessed salivary flow rate (volume/time); however, this did not affect the analysis and so the data is presented with no adjustment for flow.

ELISA methodology

Serum SARS-CoV-2 S ELISAs

SARS-CoV-2 Spike (S) specific IgGAM ELISAs (The Binding Site; TBS) were performed, as per manufacturers' instructions for serum samples, with a reported sensitivity of 98.6% and specificity of 98.3% established in healthcare workers following mild infection. This assay simultaneously detects all three antibody classes specific to SARS-CoV-2 spike protein. Results are expressed as an optical density (OD) with values ≥ 0.778 classed as positive, with the cut-off threshold generated from 689 pre-2019 serum samples. We adapted the IgGAM ELISA to assess individual SARS-CoV-2 spike specific IgG, IgA and IgM antibodies. The method was identical except for the use of sheep anti-human HRP-conjugated polyclonal antibodies against IgG (at a dilution of 1:16,000), IgA (1:2000) and IgM (1:8000) (TBS, UK). Cut offs were then individually determined for each immunoglobulin class using the method described for the IgGAM assay[18] using a frequency distribution graph of the OD results for each isotype, which was plotted from 90 pre-2019 negative serum samples. As the IgGAM kit calibrator is a combination of IgG, A, M, the individual relative proportions were assigned following assessment of the pre-2019 data and then this coefficient applied to each sample. Following this adjustment, any ratio values ≥ 1 were classed as positive as per the total IgGAM assay.

Saliva SARS-CoV-2 S ELISAs

The serum IgGAM and individual isotype ELISAs were adapted for saliva. The combined IgGAM assay was identical except for using 1:2 dilution for saliva (compared to a 1:40 dilution for serum) samples. There was no calibrator available for saliva so the cut off threshold (1.1 $OD_{450\ nm}$) was generated using data generated by testing 35 pre-2019 saliva samples, where the cut-off was defined as being greater than the highest pre-2019 sample OD.

For the individual isotype ELISAs, preliminary work with the polyclonal IgA detection antibody found non-specific binding in the pre-2019 saliva which was not found with the serum assays (data not shown), therefore the saliva individual isotype IgA detection assay was adapted and using a mouse monoclonal IgG anti-human IgA antibody MG4.156-IgA (1:4000),[19–21] whilst the polyclonal IgG and M detection antibodies were unchanged as previously described.[1] The IgA detection antibody required 1-h incubations as opposed to 30 min for the polyclonal antibodies. The thresholds

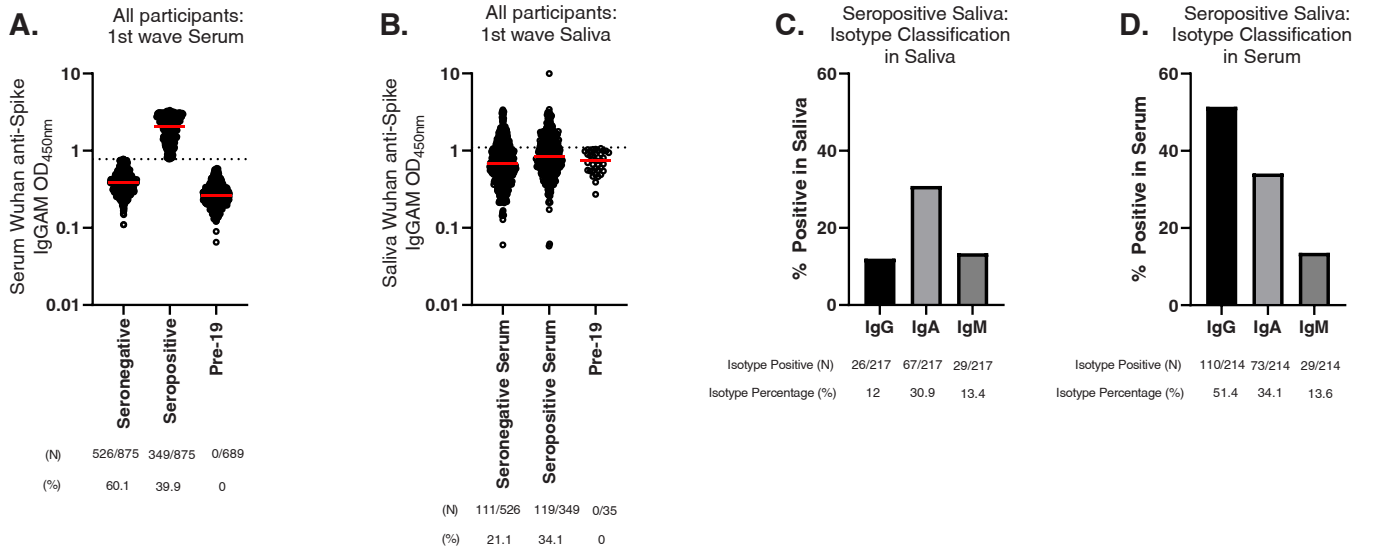

Fig. 1. Serum and salivary anti-Spike IgGAM antibodies against Wuhan strain during the first wave of the COVID-19 pandemic. A. Serum anti-Wuhan spike IgGAM antibodies in infection naïve (seronegative) and convalescent (seropositive) healthcare workers during the first wave of the COVID-19 pandemic, compared with pre-2019 (N = 689) individuals. B. Salivary anti-Spike IgGAM antibodies against the Wuhan strain and pre-2019 (N = 35) individuals were compared in the groups previously defined as seronegative and seropositive. C. Percentage of saliva IgGAM positive individuals (N = 217) that are positive for the individual IgG, IgA and IgM isotypes. D. Percentage of saliva IgGAM positive individuals (N = 214) that are positive for IgG, IgA and IgM isotypes in serum demonstrating a different distribution of antibody class in serum and saliva following natural infection. A-B. The seropositive cohort are above the dotted line. The red lines represent the median of the individuals in that cohort.

were established as before (from n = 60 pre-2019 sera); anti-Spike IgG (0.144 $OD_{450\ nm}$), IgA (0.222 $OD_{450\ nm}$), and IgM (0.085 $OD_{450\ nm}$).

Serum and saliva live SARS-CoV-2 neutralisation assay

Live virus neutralisation assays were performed using Vero cells on a subset of paired serum and saliva samples after vaccination (V2 + 6 months and after 28 days post-3rd dose of vaccination at 1:200 serum dilution and a 1:3 saliva dilution as previously described.[22] Vero cells were seeded in 96-well plates in Dulbecco's Modified Eagles Medium with 10% FBS, 1% penicillin-streptomycin, 1% L-Glutamine. SARS-CoV-2 virus (106 IU/ml stock, PHE SARS-CoV-2 England 2) at a final dilution of 1:300 was incubated with patient serum samples for 1 h at 37 °C and added to Vero cells for 48 h. The optimal dilution for serum and saliva was previously established. The cells were fixed with methanol for 5 min and stained with rabbit anti-SARS-CoV-2 clone CR3022 (Native Antigen Company, Oxford, UK) and anti-rabbit Alexaflour 555 (Thermo Fisher Scientific) and Hoechst 33420 for nuclear staining. The plates were imaged using a Thermo Cell Insight CX5 high content screening platform and custom algorithms were used to quantify percentage infection and number of viable cells. Results are expressed as percentage neutralisation with reference to pre-2019 control sera and saliva.

Statistical analysis

Data were checked for normality using the Shapiro Wilk test. Wilcoxon matched paired t-tests were performed on intra-cohort samples and Mann-Whitney U tests were performed on inter-cohort samples. The significance level was assumed a priori to be 0·05. The analyses were conducted using GraphPad Prism 9. Agreement between matched serum and saliva ELISA results was assessed by determining Spearman's rank correlation coefficient.

Results

Post-infection anti-Spike IgGAM serum and saliva antibody responses have poor agreement

The overall seroprevalence, as determined using total antibody responses against Spike protein (IgGAM antibodies) in the first wave cohort, was 39.9% (N = 349/875) (Fig. 1A), however, only 34.1% (N = 119/349) of the seropositive group also had salivary anti-spike IgGAM antibodies (Fig. 1B). There was concordance between the paired serum and saliva samples in 61% (N = 534/875) of participants (Table 2) and a weak positive correlation between serum and saliva (r = 0.21, P < 0.0001) (Supplementary Fig. 1A).

Table 2
Concordance between serum and salivary immunoglobulin levels at baseline.

Assay	Serum +/Saliva +	Serum -/Saliva +	Serum +/Saliva -	Serum -/Saliva -
Total cohort (N = 875)				
IgGAM, N (%)	119 (13.6%)	111 (12.7%)	230 (26.3%)	415 (47.4%)
Subset of patients that had tested positive saliva on the IgGAM assay, N = 213				
IgG, N (%)	22 (10.3%)	4 (1.9%)	88 (41.3%)	99 (46.5%)
IgA, N (%)	28 (13.1%)	37 (17.4%)	45 (21.2%)	103 (48.4%)
IgM, N (%)	8 (3.8%)	21 (9.9%)	21 (9.9%)	163 (76.5%)

Contingency table to describe the IgGAM concordance between serum and saliva for the entire cohort at baseline following the first wave of COVID-19 infection, prior to vaccination and concordance for the individual IgG, A and M antibodies in the subgroup of this cohort taken forward for subclass analysis as antibodies had been detected in the saliva using the IgGAM assay. Negative (-) corresponds to absence of antibodies and positive (+) to presence of relevant detectable antibodies.

Serum and salivary anti-Spike responses have a distinctive isotype distribution

In individuals who had detectable IgGAM salivary antibodies at baseline (N = 230/875, except N = 13 participants who had insufficient saliva and 17 who had insufficient serum for further testing), we measured individual immunoglobulin isotypes (IgG, IgA, and IgM) in saliva (Fig. 1C) and serum (Fig. 1D) against the whole spike protein. In saliva, IgG antibodies were detected in 12% (N = 26/217), IgA antibodies in 30.9% (N = 67/217) and IgM antibodies in 13.4% (N = 29/217) of samples. IgG antibodies were detectable in 51.4% of sera (N = 110/214), IgA antibodies in 34.1% (N = 73/214), and IgM antibodies in 13.6% (N = 29/214). There was concordance between serum and saliva for anti-spike IgG in 56.8% (N = 121/213), IgA in 61.5% (N = 131/213) and IgM in 80.3% (N = 171/213) (Table 2). There was a moderate positive correlation between the two sample matrices for IgG (r = 0.50, P < 0.0001), no correlation for IgA (r = 0.12, P = 0.07) and a weak positive correlation for IgM (r = 0.34, P < 0.0001) (Supplementary Fig. 1B-D).

Vaccination enhances salivary IgG responses independent of prior seropositivity

Individual immunoglobulin isotypes directed against the SARS-CoV-2 spike glycoprotein were analysed in 66 paired serum and saliva samples at baseline (at time of study recruitment) and post BNT162B2 vaccination at 28 days post V2 (V2 + 28) (N = 37 seropositive and N = 29 seronegative). In 20 of these subjects, it was possible to also obtain additional paired samples at 28 days post V3 (V3 + 28) (N = 11 seropositive and N = 9 seronegative).

Similar to previous studies, we found previously seropositive HCWs had significantly higher serum anti-Spike IgG compared to those that were previously seronegative (Fig. 2A) following their second (8.07 v 5.26 ratio) and third vaccination (4.29 v 4.20 ratio). 86.5% of previously seropositive individuals had detectable serum IgG, rising to 100% post V2 and V3 and 0% of previously seronegative individuals at baseline increasing to 100% post V2 and 100% post V3 (Table 3). For saliva, there was no significant difference in the IgG level following vaccination (Fig. 2B). Following 2 doses of vaccine, 70.3% of previously seropositive and 65.5% of previously seronegative individuals had salivary IgG antibodies and following 3 doses 90.9% of previously seropositive individuals and 88.9% of previously seronegative individuals had salivary IgG antibodies (Table 4). Overall concordance for anti-Spike IgG antibodies between serum and saliva post-vaccination (post V2 + V3) was 73.2% (N = 63/86) (Table 5) with no correlation (r = −0.08 P = 0.45, Supplementary Fig. 2B).

Previously seropositive HCWs had significantly higher serum anti-spike IgA levels than seronegative HCWs following their second (4.16 v 1.59 ratio) and third vaccinations (8.42 v 2.92 ratio) (Fig. 2C). 40.5% of previously seropositive individuals had detectable serum IgA at baseline increasing to 91.9% post V2 and 100% post V3 and 3.45% of seronegative individuals at baseline increasing to 82.8% post V2 and 88.9% post V3 (Table 3). For saliva, there was no significant difference in the IgA level following vaccination (Fig. 2D). Following 2 doses of vaccine, 46.0% of previously seropositive and 55.2% of previously seronegative individuals had salivary IgA antibodies and following 3 doses 27.2% of previously seropositive individuals and 22.2% of seronegative individuals had salivary IgA antibodies (Table 4). Concordance for anti-Spike IgA antibodies between serum and saliva post vaccination (V2 +V3) was 45.3% (N = 39/86) (Table 5) with no correlation (r = 0.12, P = 0.28, Supplementary Fig. 2D).

Previously seropositive HCWs had significantly higher serum anti-spike IgM levels than seronegative HCWs following their second (0.39 v 0.39 ratio) and third vaccinations (0.80 v 0.78 ratio) (Fig. 2E). 13.5% of previously seropositive individuals had detectable serum IgM at baseline changing to 8.1% post V2 and 36.4% post V3 and 0% of seronegative individuals at baseline increasing to 0% post V2 and 33.3% post V3 (Table 3). For saliva, there was no significant difference in the IgM level following vaccination (Fig. 2F). Following 2 doses of vaccine, 5.4% of previously seropositive and 6.9% of previously seronegative individuals had salivary IgM antibodies and following 3 doses 36.4% of previously seropositive individuals and 44.4% of seronegative individuals had salivary IgM antibodies (Table 4). Concordance for anti-Spike IgM antibodies between serum and saliva post vaccination (V2 + V3) was 84.9% (N = 73/86) (Table 5) with a moderate correlation r = 0.51 0.6 P < 0.0001, Supplementary Fig. 2F).

Neutralising serum and salivary antibodies following intramuscular vaccination

We evaluated the neutralisation ability of antibodies generated in 49 of the 66 paired serum and saliva samples at 6 months post 2 doses of BNT162B2 and at least 28 days post-third dose of BNT162B2 (pre and post 3rd vaccination). We show data, combining both seronegative and seropositive cohorts as no significant difference was found in salivary antibodies at V2 + 6 months nor V3 time points.

Of the serum samples, 87.8% (43/49) exhibited neutralising activity (>50% neutralisation) at V2 + 6 months and this rose to 100% (49/49) post V3 (median % neutralisation 87.1% v 100%, P < 0.0001) (Fig. 3A). Only 5 of 49 (10.2%) saliva samples had a neutralisation activity of > 50% at the V2 + 6-month time-point and this increased to 15 out of 49 (30.6%) post V3 (median 1% v 21.4% P = 0.0014) (Fig. 3B).

There is significantly more neutralisation capability from antibodies in serum compared with saliva post V2 + 6 months (87.1 v 1%, p < 0.0001) and V3 (100% v 21.4%, p < 0.0001). Comparison of paired serum and saliva (N = 49) demonstrated no correlation either at the V2 + 6 month (r = 0.033 [95% CI −0.259 to 0.319]; p = 0.824) (Fig. 3C) or post V3 time point r = 0.010 [−0.279–0.298]; p = 0.945) (Fig. 3D).

Discussion

This longitudinal study of paired serum and saliva samples has established that there are significant differences in the characteristics of antibody responses in serum and saliva following SARS-CoV-2 infection and PFZ vaccination. To our knowledge, this is the largest investigation to date of paired systemic and oral antibody responses. Overall, findings suggest that saliva cannot be used interchangeably with serum as a seroprevalence tool, either post-intramuscular vaccination or infection.

Saliva is preferred by health care workers as an attractive medium for seroprevalence testing as it can be self-collected, is non-invasive and cheaper to acquire.[23] To explore the use of saliva to detect SARS-CoV-2 antibodies, we tested paired serum and saliva samples using a high sensitivity combined IgGAM assay. Despite the assay measuring IgG and IgA, we found only modest overall concordance. There was a weak but significant correlation between the serum and saliva IgGAM results at baseline. When the individual isotypes were examined, there was a moderate correlation for IgG and a weak correlation for IgM but no correlation for IgA. We show that SARS-CoV-2 antibodies can be detected with relative ease in saliva when employing high sensitivity assays; however, salivary and serum antibody patterns do differ and there is reduced sensitivity for saliva. In response to vaccination, we show that salivary IgG and IgA levels rise and that these antibody levels can be boosted through vaccination. However, unlike in serum,[16] we found that previous infection does not enhance salivary IgG or IgA vaccine responses. The explanation for this finding is not known but highlights the

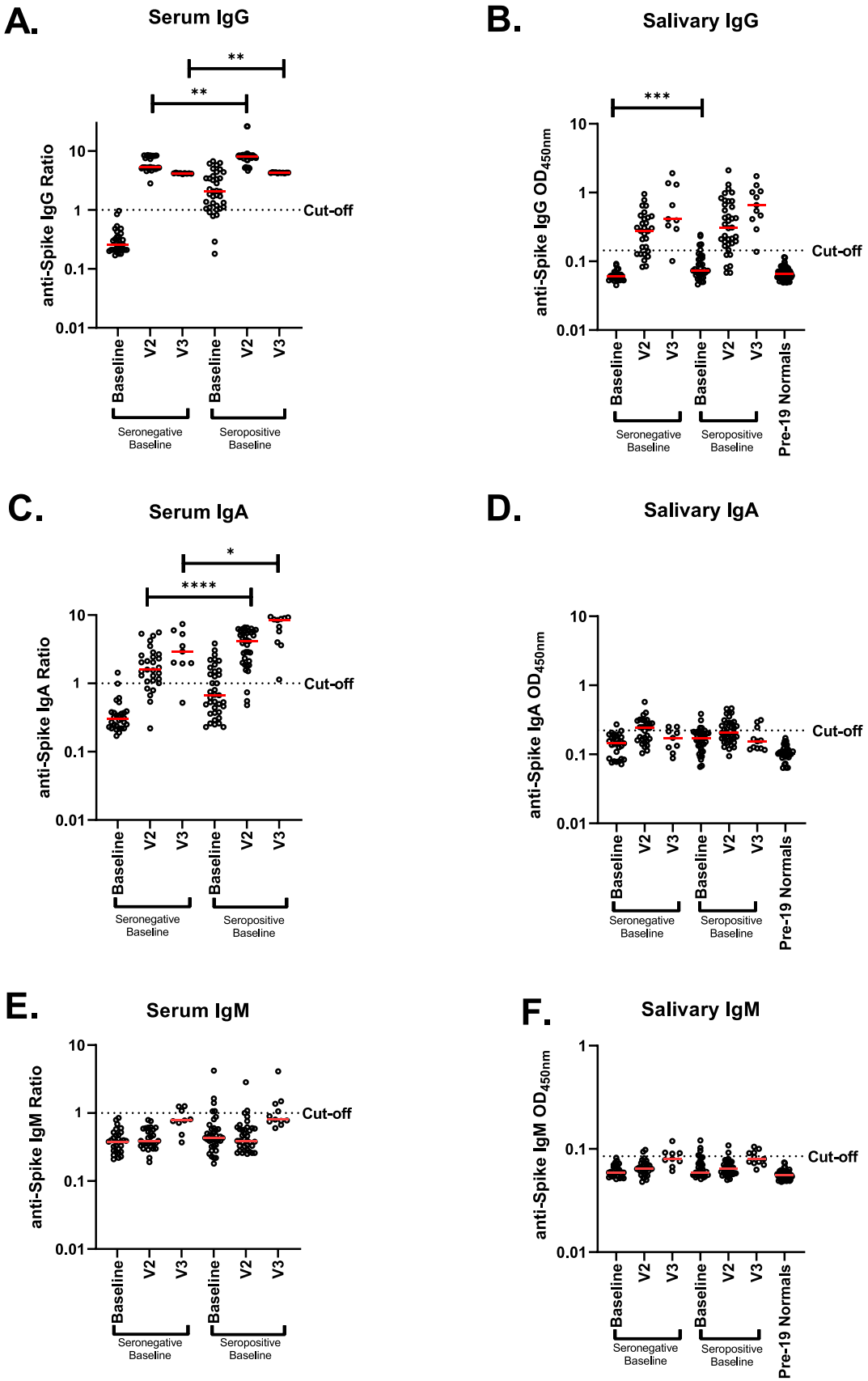

Fig. 2. Serum and salivary anti-Spike IgG, IgA, and IgM antibodies against Wuhan strain following SARS-CoV-2 spike vaccination. Anti-Wuhan spike IgG responses to SARS-CoV-2 Pfizer BioNTech vaccines in both Seronegative and Seropositive cohorts at time of vaccination in serum (A) and saliva (B). Saliva collected from pre-2019 are included for comparison. Similarly for IgA in serum (C) and saliva (D) and IgM serum (E) and saliva (F). Results are given for pre (Baseline) and post-second (V2) (V2 + 28 days) and third dose (V3) (V3 + 28 days) of vaccination. The cohort that are considered seropositive are included above the dotted line. The red line represents the median of the individuals in that cohort. Significance is indicated as follows: *p ≤ 0.05, **p ≤ 0.01, ***p < 0.001, ****p < 0.0001.

Table 3
Serum anti-Spike IgG, IgA, and IgM in previously seronegative and seropositive health care workers before and after vaccination.

Serum	Timepoint	Previously Seronegative (Median Ratio (range))	Previously Seronegative (Percent Positive, %, N)	Previously Seropositive (Median Ratio (range))	Previously Seropositive (Percent Positive, %, N)	P-value
IgG	Baseline	0.26 (0.17–0.96)	0% (N = 0/29)	2.06 (0.18–6.69)	86.5% (N = 32/37)	***P < 0.0001,*** ****
	V2	5.26 (2.81–8.52)	100% (N = 29/29)	8.07 (4.63–26.2)	100% (N = 37/37)	***P = 0.0034,*** ***
	V3	4.20 (4.05–4.28)	100% (N = 9/9)	4.29 (4.18–4.39)	100% (N = 11/11)	***P = 0.0022,*** ***
IgA	Baseline	0.30 (0.17–1.43)	3.45% (N = 1/29)	0.67 (0.23–3.83)	40.5%(N = 15/37)	***P < 0.0001,*** ****
	V2	1.59 (0.22–5.56)	82.8%(N = 24/29)	4.16 (0.48–6.58)	91.9%(N = 34/37)	***P < 0.0001,*** ****
	V3	2.92 (0.52–7.41)	88.9% (N = 8/9)	8.42 (1.14–9.39)	100% (N = 11/11)	***P = 0.0125,*** *
IgM	Baseline	0.38 (0.21–0.85)	0% (N = 0/29)	0.43 (0.18–4.20)	13.5% (N = 5/37)	P = 0.09
	V2	0.39 (0.19–0.79)	0% (N = 0/29)	0.39 (0.25–2.84)	8.1% (N = 3/37)	P = 0.8
	V3	0.78 (0.37–1.26)	33.3% (N = 3/9)	0.80 (0.60–4.10)	36.4% (N = 4/11)	P = 0.5

IgG, A and M antibodies levels are reported at baseline and following 2 (V2) and 3 (V3) doses of Pfizer BioNTech 162B2 vaccine for individuals that were seropositive and seronegative at baseline during the first COVID-19 infection wave spring/summer 2020. Antibody levels are reported as medians and range, with P-values from Mann-Whitney U tests (unpaired). Bold and italicised P-values are significant at P < 0.05. PFZ: Pfizer BioNTech 162B2 vaccine. Significance is indicated as follows: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. The percentage of the seronegative or seropositive population are also reported as a proportion of the group size.

Table 4
Saliva anti-Spike IgG, IgA, and IgM in previously seronegative and previously seropositive health care workers before and after vaccination.

Saliva	Timepoint	Previously Seronegative (Median OD450nm (Min-Max))	Previously Seronegative (Percent Positive, %, N = X/X)	Previously Seropositive (Median OD450nm (Min-Max))	Previously Seropositive (Percent Positive, %, N = X/X)	P-value
IgG	Baseline	0.06 (0.05–0.09)	0% (N = 0/0)	0.07 (0.05–0.244)	13.5% (N = 5/37)	***P = 0.0003,*** ***
	V2	0.28 (0.08–0.95)	65.5%(N = 19/29)	0.31 (0.07–2.11)	70.3%(N = 26/37)	P = 0.3
	V3	0.41 (0.10–1.91)	88.9% (N = 8/9)	0.66 (0.14–1.73)	90.9% (N = 10/11)	P = 0.8
IgA	Baseline	0.14 (0.07–0.27)	10.3% (N = 3/29)	0.17 (0.07–0.39)	32.4%(N = 12/37)	P = 0.07
	V2	0.24 (0.10–0.57)	55.2%(N = 16/29)	0.21 (0.09–0.47)	46.0%(N = 17/37)	P = 0.9
	V3	0.17 (0.09–0.25)	22.2% (N = 2/9)	0.15 (0.12–0.20)	27.2% (N = 3/11)	P = 0.9
IgM	Baseline	0.059 (0.051–0.082)	0% (N = 0/29)	0.059 (0.051–0.121)	10.8% (N = 4/37)	P = 0.3
	V2	0.064 (0.048–0.098)	6.9% (N = 2/29)	0.064 (0.050–0.108)	5.4% (N = 2/37)	P = 0.9
	V3	0.080 (0.061–0.119)	44.4% (N = 4/9)	0.080 (0.063–0.105)	36.4% (N = 4/11)	P = 0.9

IgG, A and M antibodies levels are reported at baseline and following 2 (V2) and 3 (V3) doses of Pfizer BioNTech 162B2 vaccine for individuals that were seropositive and seronegative at baseline during the first COVID-19 infection wave spring/summer 2020. Antibody levels are reported as medians and range, with P-values from Mann-Whitney tests U(unpaired). Bold and italicised P-values are significant at P < 0.05. PFZ: Pfizer BioNTech 162B2 vaccine. Significance is indicated as follows: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. The percentage of the seronegative or seropositive population are also reported as a proportion of the group size.

Table 5
Concordance between serum and salivary immunoglobulin levels following COVID-19 vaccination.

Vaccination (post-V2+V3) N = 86	Serum +/Saliva +	Serum -/Saliva +	Serum +/Saliva -	Serum -/Saliva -
IgG	63 (73.2%)	0 (0%)	23 (26.7%)	0 (0%)
IgA	34 (39.5%)	4 (4.7%)	43 (50%)	5 (5.8%)
IgM	3 (3.5%)	8 (9.3%)	7 (8.1%)	70 (81.4%)

Contingency table to describe individual immunoglobulin isotypes following vaccination with 2 (V2) and 3 (V3) doses of SARS-CoV-2 Pfizer BioNTech. As there was no difference in the salivary responses between V2 and V3, this analysis has been combined. Negative (-) corresponds to absence of antibodies and positive (+) to presence of relevant detectable antibody.

compartmentalisation of the mucosal lymphatics and potential limitations of an intramuscular vaccination.

We also found that saliva was less sensitive in detecting IgG following vaccination than serum. This confirms data from other studies that have measured vaccination responses in saliva.[24] Characterising the antibody response post-vaccination found that salivary IgG was more prevalent than IgA following vaccination, whereas IgA predominated following infection. This differs however from a previous study which found similar amounts of salivary IgG and IgA post-vaccination; this may be explained by different ELISA platforms or methods of collecting oral fluids.[25] The individual positivity rate for separate IgG, IgA and IgM ELISAs was lower than the combined IgGAM assay reflecting the enhanced sensitivity of the combined ELISA which has been previously reported.[2,15,18]

The source of salivary IgG and IgM is contentious and is thought to be partially produced in local lymph nodes but also derived from the circulation through gingival crevicular fluid entering into saliva. Distinguishing antibodies derived from these two sources is not straight forward.[26] One approach is to examine samples from monoclonal gammopathies where neoplastic plasma cells in bone marrow secrete large amounts of monoclonal immunoglobulins that can be easily distinguished from polyclonal immunoglobulin. A recent study of paired serum and saliva samples from myeloma patients, demonstrates that very little salivary immunoglobulin is blood derived. This apparent partitioning of systemic and oral immunity has important implications when considering route of vaccination.[18]

Serum and salivary IgM levels were low throughout baseline and post-vaccination. Possible explanations include the fact that IgM declines faster than IgG[26,27] and our sampling window was after the peak of 20 days previously reported. Also, the overall amount of IgM in saliva is lower than IgG or IgA (IgM 4.1 mg/L, IgA 140 mg/L and IgG 16 mg/L).[28] An alternative hypothesis is that previous exposure to other human coronaviruses could be affecting IgM responses. Ruggiero et al. has previously reported that not all individuals make an IgM response to SARS-CoV-2 vaccination and propose that there is

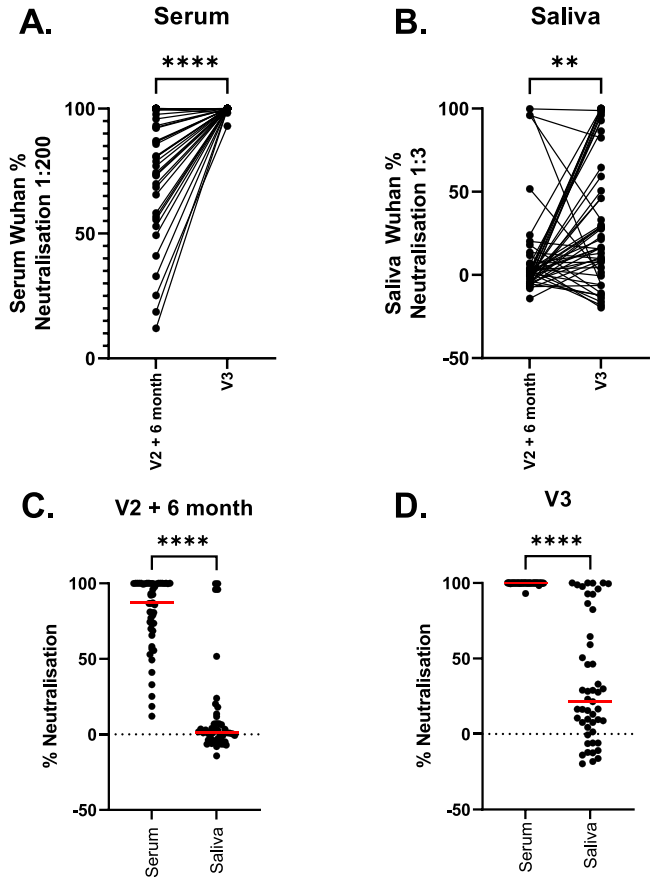

Fig. 3. Serum and saliva antibody neutralisation against the Wuhan strain. A) Serum and B) Saliva neutralisation percentages at V2 + 6 month and V3 + 28 days timepoints (comparison of pre and post third dose of SARS-CoV-2 Pfizer BioNTech vaccine). Comparison of serum and saliva neutralisation percentages at C) V2 + 6 month and D) V3 post- SARS-CoV-2 Pfizer BioNTech vaccines. Significance is indicated as follows: *p ≤ 0.05, **p ≤ 0.01, ***p < 0.001, ****p < 0.0001.

preferential boosting of cross-reactive immunity resulting in a blunted spike specific SARS-CoV-2 IgM response.[29]

Salivary IgA is predominantly secreted in a dimeric form, generated by local plasma cells found in the stroma of salivary glands.[25] It has previously been reported that the serum IgA response following infection appears to be short-lived in the saliva.[11,12] Therefore, differences between serum and saliva in relation to natural infection may also be due to kinetics and other studies have found salivary IgG detectable up to 9 months whereas IgA maybe detectable for only up to 2 or 3 months.[2,8,10] However, we found no difference in the likelihood of antibody positivity with time from symptom onset, in serum nor saliva, suggesting the sampling time frame was short enough to not affect the analysis. As the predominant immunoglobulin class in the saliva is IgA, this waning will have a larger effect on saliva than serum samples in a combined immunoglobulin detection assay. There was no fixed point for sampling in the first wave and therefore, it is possible that antibody decay could have contributed to the poor concordance between specimens. Given the importance of salivary antibodies in the first line of defence against antigens that enter via oral mucosal surfaces, these findings may allude to the observed differences in protective versus sterilising immunity. Indeed, serum and saliva antibody differences were not only limited to quantitation but also to neutralisation function in our study.

It is also important to consider that while biological differences are likely largely responsible for differences between specimens, methodological factors may have contributed. The finding of serum negative/saliva positive participants in the first wave could be explained by a local mucosal immune response which does not subsequently stimulate a systemic immune response. Alternatively, the use of a polyclonal detection antibody may result in cross-reactivity and false positives. The finding of poly-reactive IgA in human secretions has been described previously.[12] However, higher specificity in SARS-CoV-2 ELISAs has been reported[30] and we also found a lower detection rate in the individual isotype ELISAs where a monoclonal detection antibody was used. Standardising approaches to antibody detection have been highlighted prior to[31] and during the COVID-19 pandemic where cross-reactivity, batch-variability, and wrong applications have been considered.[32]

In conclusion, functional salivary antibodies are detectable following SARS-CoV-2 infection and vaccination but with different isotypes being generated in the serum and saliva. However, the low sensitivity of saliva, compared with serum, makes it unsuitable for seroprevalence studies and they are not interchangeable. Collectively, these findings should be considered when devising next generation vaccines.

Declaration of Competing Interest

AC is employed by The Binding Site Group Ltd. The SARS-CoV-2 ELISA was developed and commercialised between The Binding Site Group Ltd and the Clinical Immunology Service at University of Birmingham (including AR, SF, MTD, AS, AC). The rest of the authors declared no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2023.07.018.

References

1. Shields A, Faustini SE, Perez-Toledo M, Jossi S, Aldera E, Allen JD, et al. *SARS-CoV-2 seroprevalence and asymptomatic viral carriage in healthcare workers: a cross-sectional study. Thorax* 2020;**75**:1089–94.
2. Shields AM, Faustini SE, Perez-Toledo M, Jossi S, Allen JD, Al-Taei S, et al. *Serological responses to SARS-CoV-2 following non-hospitalised infection: clinical and ethnodemographic features associated with the magnitude of the antibody response. BMJ Open Respir Res* 2021;**8**(1).
3. Houlihan CF, Vora N, Byrne T, Lewer D, Kelly G, Heaney J, et al. *Pandemic peak SARS-CoV-2 infection and seroconversion rates in London frontline health-care workers. Lancet* 2020;**396**(10246):e6–7.

4. Eyre DW, Lumley SF, O'Donnell D, Campbell M, Sims E, Lawson E, et al. *Differential occupational risks to healthcare workers from SARS-CoV-2 observed during a prospective observational study. eLife* 2020;**9**:1–21.
5. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. *Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med* 2021;**27**(7):1205–11.
6. England P. *SARS-CoV-2 variants of concern and variants under investigation in England. Tech Brief* 2021;**12**:1–53.
7. Pisanic N, Randad PR, Kruczynski K, Manabe YC, Thomas DL, Pekosz A, et al. *COVID-19 serology at population scale: SARS-CoV-2-specific antibody responses in saliva. J Clin Microbiol* 2020;**59**(1):e02204–20.
8. Isho B, Abe KT, Zuo M, Jamal AJ, Rathod B, Wang JH, et al. *Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. Sci Immunol* 2020;**5**(52):eabe5511.
9. Gudbjartsson DF, Norddahl GL, Melsted P, Gunnarsdottir K, Holm H, Eythorsson E, et al. *Humoral immune response to SARS-CoV-2 in Iceland. N Engl J Med* 2020;**383**(18):1724–34.
10. Alkharaan H, Bayati S, Hellström C, Aleman S, Olsson A, Lindahl K, et al. *Persisting salivary IgG against SARS-CoV-2 at 9 months after mild COVID-19: a complementary approach to population surveys. J Infect Dis* 2021;**224**(3):407–14.
11. Piano Mortari E, Russo C, Vinci MR, Terreri S, Fernandez Salinas A, Piccioni L, et al. *Highly specific memory B cells generation after the 2nd dose of BNT162b2 vaccine compensate for the decline of serum antibodies and absence of mucosal IgA. Cells* 2021;**10**(10):2541.
12. Quan CP, Berneman A, Pires R, Avrameas S, Bouvet JP. *Natural polyreactive secretory immunoglobulin A autoantibodies as a possible barrier to infection in humans. Infect Immun* 1997;**65**(10):3997–4004.
13. Pinilla YT, Heinzel C, Caminada L-F, Consolaro D, Esen M, Kremsner PG, et al. *SARS-CoV-2 antibodies are persisting in saliva for more than 15 months after infection and become strongly boosted after vaccination. Front Immunol* 2021;**12**:1–7.
14. Silva J, Lucas C, Sundaram M, Israelow B, Wong P, Klein J, et al. Saliva viral load is a dynamic unifying correlate of COVID-19 severity and mortality. medRxiv; 2021.
15. Faustini SE, Jossi SE, Perez-Toledo M, Shields AM, Allen JD, Watanabe Y, et al. *Development of a high-sensitivity ELISA detecting IgG, IgA and IgM antibodies to the SARS-CoV-2 spike glycoprotein in serum and saliva. Immunology* 2021;**164**(1):135–47.
16. Payne RP, Longet S, Austin JA, Skelly DT, Dejnirattisai W, Adele S, et al. *Immunogenicity of standard and extended dosing intervals of BNT162b2 mRNA vaccine. Cell* 2021;**184**(23):5699–714. e11.
17. Heaney JLJ, Faustini S, Evans L, Rapson A, Collman E, Emery A, et al. *Investigating the utility of saliva immunoglobulins for the detection of myeloma and using myeloma proteins to clarify partition between oral and systemic immunity. Eur J Haematol* 2022;**108**(6):493–502.
18. Cook AM, Faustini SE, Williams LJ, Cunningham AF, Drayson MT, Shields AM, et al. *Validation of a combined ELISA to detect IgG, IgA and IgM antibody responses to SARS-CoV-2 in mild or moderate non-hospitalised patients. J Immunol Methods* 2021;**494**:113046.
19. Lund J, Takahashi N, Pound JD, Goodall M, Jefferis R. *Multiple interactions of IgG with its core oligosaccharide can modulate recognition by complement and human Fc gamma receptor I and influence the synthesis of its oligosaccharide chains. J Immunol* 1996;**157**(11):4963–9.
20. Grafton G, Goodall M, Gregory CD, Gordon J. *Mechanisms of antigen receptor-dependent apoptosis of human B lymphoma cells probed with a panel of 27 monoclonal antibodies. Cell Immunol* 1997;**182**(1):45–56.
21. Farris MA, Hardie D, De Lange G, Jefferis DR. *Immunogenic and antigenic epitopes of immunoglobulins X: monoclonal antibodies specific for human IgA, the IgA1 and IgA2 subclasses and an nA2m(2) iso-allotypic epitope. Vox Sang* 1985;**48**(2):116–21.
22. Shields AM, Faustini SE, Hill HJ, Al-Taei S, Tanner C, Ashford F, et al. *SARS-CoV-2 vaccine responses in individuals with antibody deficiency: findings from the COV-AD study. J Clin Immunol* 2022;**42**(5):923–34.
23. Shields AM, Brown H, Phillips N, Drayson MT, Richter AA, Richter AG. *Health care professionals' confidence and preferences for diagnostic assays for SARS-CoV-2: a global study. Front Public Health* 2021;**9**:569315.
24. Katz MJ, Heaney CD, Pisanic N, Smith L, Bigelow BF, Sheikh F, et al. *Evaluating immunity to SARS-CoV-2 in nursing home residents using saliva IgG. J Am Geriatr Soc* 2022;**70**(3):659–68.
25. Brandtzaeg P. *Secretory immunity with special reference to the oral cavity. J Oral Microbiol* 2013;**5**:1–24.
26. Jin Y, Wang M, Zuo Z, Fan C, Ye F, Cai Z, et al. *Diagnostic value and dynamic variance of serum antibody in coronavirus disease 2019. Int J Infect Dis* 2020;**94**:49–52.
27. Long Q-X, Liu B-Z, Deng H-J, Wu G-C, Deng K, Chen Y-K, et al. *Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med* 2020;**26**(6):845–8.
28. Grönblad EA. *Concentration of immunoglobulins in human whole saliva: effect of physiological stimulation. Acta Odontol Scand* 1982;**40**(2):87–95.
29. Ruggiero A, Piubelli C, Calciano L, Accordini S, Valenti MT, Carbonare LD, et al. *SARS-CoV-2 vaccination elicits unconventional IgM specific responses in naïve and previously COVID-19-infected individuals. eBioMedicine* 2022;**77**:103888.
30. Costantini VP, Nguyen K, Lyski Z, Novosad S, Bardossy AC, Lyons AK, et al. *Development and validation of an enzyme immunoassay for detection and quantification of SARS-CoV-2 salivary IgA and IgG. J Immunol* 2022;**208**(6):1500–8.
31. Baker M. *Reproducibility crisis: blame it on the antibodies. Nature* 2015;**521**(7552):274–6.
32. National Academies of Sciences, Engineering, and Medicine. *Rapid expert consultation on SARS-CoV-2 laboratory testing for the Covid-19 pandemic (April 8, 2020).* Rapid expert consultations on the COVID-19 pandemic: March 14, 2020–April 8, 2020. US: National Academies Press; 2020.