Salivary antibodies are detected with a commercial anti-SARS-CoV-2 assay only after two doses of vaccine using serum thresholds

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A B S T R A C T

Salivary matrix is an appealing specimen type for SARS-CoV-2 serology because of ease of collection and potential for concurrent nucleic acid testing. We address the feasibility of salivary matrix to detect anti-SARS-CoV-2 antibodies using two commercially available anti-SARS-CoV-2 Total antibody assays including analytical validations.

Matched serum and saliva samples were collected from 10 convalescent COVID-19 patients and tested using a quantitative anti-Spike Total antibody assay and a qualitative anti-Nucleocapsid Total antibody assay from Roche Diagnostics. Both assays were 100% sensitive for COVID-19 history in serum. However, saliva samples were below serum positivity thresholds. We then collected longitudinal salivary samples from a volunteer cohort receiving the Pfizer-BioNTech COVID-19 BNT162b2 vaccine. Saliva was negative for anti-SARS-CoV-2 antibodies at 5 time points after a single dose of vaccine including day 56 when mean (min-max) serum levels of anti-Spike Total antibody were 79.0 U/mL (46.6–110.1) (N = 8). After a second vaccine dose serum-matched samples were beyond the analytical measuring range of the assay (>2500 U/mL), and detection of salivary anti-Spike Total antibody was achieved in all volunteers (12.2 U/mL [2.0–32.7]) (N = 11) 30 days after the second dose. Mean anti-Spike Total antibody levels in serum (1558 U/mL (434–>2500)) and saliva (2.6 U/mL (<0.4–11.4)) declined 216–233 days after the first dose of vaccine (P < 0.05); and saliva was 75% sensitive for two doses of vaccination at this latter time point (N = 25). These data suggest commercial assays are capable of detecting vaccine status after two doses of BNT162b2 vaccine up to 6 months and could inform COVID-19 surveillance.

1. Introduction

Detection of salivary anti-SARS-CoV-2 antibodies has been reported in the convalescent period and after vaccination [1–9]. This proof of concept is important because saliva is more convenient and less invasive than blood collection, and could have a role for epidemiologic purposes or at points of entry. Indeed, a single saliva specimen could allow testing for anti-SARS-CoV-2 antibodies, SARS-CoV-2 antigen, and viral RNA in the same collection. Furthermore, the relevance of salivary antibodies in the oro-/nasopharyngeal cavity is they may be implicit in protecting against infection from respiratory pathogens [2,10]. And because infectious virus is present in the saliva of symptomatic and asymptomatic individuals, the presence of salivary antibody could plausibly reduce viral infection and transmission [10]. Intramuscular vaccines such as BNT162b2 primarily elicit serum IgG production [11], although there is

a clear role of IgA in the early response to vaccine and infection with more potent neutralizing properties than IgG [12,13]. Therefore, there is likely a role for IgA and IgG in combating SARS-CoV-2 infections, and rodent models show that IgG transudates in nasal fluid reduce shedding of other respiratory viruses, albeit to a lesser degree than IgA [14]. There is presently little data on the performance of commercially available anti-SARS-CoV-2 assays to detect salivary antibodies [4], which is important because they are widely available, high-throughput, and scalable. To this end, we aimed to test for salivary antibodies in recovered COVID-19 patients as well as longitudinally among vaccinated volunteers using the two commercially available anti-SARS-CoV-2 Total Antibody assays.

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2. Materials and methods

2.1. Subjects

Matched serum and saliva samples were collected from 10 patients for seroepidemiologic purposes on Prince Edward Island, Canada. The patients had a history of RT-PCR confirmed COVID-19 within 3 months of collection, and 2/10 patients were hospitalized during infection. All saliva samples were collected using "Salivettes" based on manufacturer instructions (Sarstedt, Germany).

Saliva samples were collected longitudinally from a cohort of volunteers inoculated with a similar course of Pfizer-BioNTech COVID-19 BNT162b2 vaccine. Baseline saliva samples (N = 10) were collected prior to vaccination (day 0) and on days 2, 7, 14, and 30 (N = 8) after a single dose of vaccine. Matched serum and saliva sample were then collected prior to a second dose of BNT162b2 on day 56 (N = 8), day 70 (N = 8), and day 86 (N = 11). Study day 86 included 4 additional cohort volunteers on the same vaccine dosing schedule not collected at previous time points. Note that study days 70 and 86 correspond to 14 and 30 days after the second dose of vaccine, respectfully. A final collection included 25 volunteers 216–233 days (N = 12 on day 231, N = 7 on day 218, N = 2 on day 228, N = 1 on days 216, 229, 230, 233) after the first dose of vaccine, which corresponds to approximately 6 months after the second BNT162b2 dose. The collection days 216–233 included samples from 7 individuals collected at earlier time points plus 18 additional cohort volunteers. Collections were cleared by the Health PEI research ethics board.

2.2. Assays

All saliva and serum specimens were measured using anti-SARS-CoV-2 assays available from Roche Diagnostics. The assays detect antibodies specific for either the SARS-CoV-2 nucleocapsid protein (anti-Nuc Total Ab) in a qualitative format relative to a manufacturer recommended cut-off index, or the SARS-CoV-2 spike protein (anti-Spike Total Ab) in a quantitative format reported in U/mL. Saliva was measured and interpreted based on serum thresholds on cobas e601 or e801. Linearity was assessed by mixing the highest positive saliva sample with blank saliva, and assessed by regression analysis measured 4-times at each dilution. Within-run and between-run precision was calculated measuring positive and negative saliva samples consecutively 10-times for a within-run calculation and 5-times for 5-days for between-run calculation. Recovery was assessed through dilution of calibrator into blank saliva with an acceptability limit of 5%. Stability was evaluated at 4 °C for 6 months and a 2-times freeze-thaw cycle.

2.3. Statistics

We report the anti-Nuc Total Ab as positive or negative; and present the anti-Spike Total Ab values as a mean with minimum and maximum values in parenthesis. Time-course values were compared using unpaired or paired t-tests. Regression analysis was used to compare matched serum and saliva levels and to assess linearity. $P < 0.05$ was considered significant. Statistics were performed using Prism 9.3.1 (San Diego, CA).

3. Results

3.1. Analytical validation of anti-Spike Total Ab assay in salivary matrix

The anti-Spike Total Ab assay was linear to 38.0 U/mL in saliva (y = 1.0x + 0.5; R^2 = 1.0), and spiked samples recovered 98% of expected target. The within-run and between-run precision was 1.7% and 0.6% at low (mean: 0.8 U/mL) and high levels (mean: 38.1 U/mL), respectively. Between-run precision was consistently < 0.4 U/mL in a blank sample and 3.6% in positive samples (mean: 7.7 U/mL). All samples remained positive after 6 months at 4 °C, although levels declined 34% during this time span (mean bias: −3.8 U/mL). Samples were within 0.5% of original concentration after two freeze thaw cycles.

3.2. anti-SARS-CoV-2 Total antibodies in recovered COVID-19 patients

Both anti-SARS-CoV-2 assays were 100% sensitive for COVID-19 history in recovered patient serum although all saliva samples were negative for anti-SARS-CoV-2 Total antibodies (Table 1).

3.3. Longitudinal anti-SARS-CoV-2 antibodies among vaccinated volunteers

Salivary levels of anti-Spike Total Ab and anti-Nuc Total Ab were undetectable up to 56 days after a single dose of vaccine (Table 1; N = 8). On day 56, all volunteers were positive with the anti-Spike Total Ab assay in serum (N = 8; Table 1), and levels at this time point did not differ from recovered COVID-19 patient serum (P = 0.2). After two doses of BNT162b2, serum anti-Spike Total Ab levels were greater than detection (>2500 U/mL) on study days 70 and 86 (Table 1). Importantly, saliva samples were positive for anti-Spike Total Ab on day 70 and 86, and mean values did not change (P = 0.1) within-individuals nor between-individuals during this time span (Table 1). On days 216–233 the anti-Spike Total Ab assay was only 75% sensitive for double vaccination in saliva using serum thresholds, although anti-Spike Total Ab levels were detectable (>0.4 U/mL) in 23/25 saliva specimens. All individuals remained positive in serum on days 216–233, although mean levels of anti-Spike Total Ab declined (P < 0.05) in serum and saliva versus day 86 (Table 1). There was no correlation between serum and saliva anti-Spike Total Ab levels on days 216–233 (P = 0.9). All vaccinated individuals were negative for the anti-Nuc Total Ab assay in serum and saliva at all study time points, and anti-Nuc Total Ab was not measured in saliva on days 216–233.

4. Discussion

Salivary detection of anti-SARS-CoV-2 antibody may provide an important tool for the purposes of episurveillance and pharmacovigilance. While the kinetics of the systemic response to infection [15] and vaccination are well described [16], there is less data describing the salivary antibody kinetics upon inoculation with mRNA vaccines. Indeed, we report the presence of anti-SARS-COV-2 antibodies in saliva up to 30 days after two doses of the BNT162b2 vaccine using commercial assays and serum positivity thresholds, and up to 6 months in 75% of vaccinated volunteers. Furthermore, salivary antibody detection was only achieved in the presence of elevated serum anti-Spike Total Ab (≥434 U/mL), although serum and saliva antibody levels were not correlated. The analytical performance of the anti-Spike Total Ab assay was excellent and serves as a proof of concept for other clinical laboratories considering salivary matrix for SARS-CoV-2 antibody testing.

Salivary antibodies were 100% sensitive for two-doses of BNT162b2 vaccine for 30 days and the sensitivity was reduced to 75% after 6 months, which could suggest waning immunity during this time period. Among the five false negative volunteers 1 was immunocompromised and on methotrexate [17], 1 was 32 weeks pregnant, 2 have family history of autoimmune disease, and the other had no relevant history. Neutralization assays are presently the only known correlate of immunity [18], although it is debatable whether immunity is achievable and dynamic for SARS-CoV-2 in the face of excessive viral load challenge, and the emergence of novel variants. Regardless, plaque reduction neutralization assays are the gold standard but their widespread availability is low and were not measured here. There is evidence that salivary antibodies do elicit neutralization of Vesicular Stomatitis virus expressing wild-type spike protein, and similar to our findings neutralization properties of saliva declined 6 months after inoculation with BNT162b2 [7,8]. Crucially, waning humoral immunity has been shown

Table 1

Anti-Spike Ab and anti-Nuc Ab measurements in serum and saliva of recovered COVID-19 patients and vaccinated volunteers. The anti-Spike Ab quantitative assay is presented as mean (min-max) in serum and saliva, and the anti-Nuc Assay is reported as positive or negative.

Subject Type	N	Serum		Saliva	
		anti-Spike Total Ab (U/mL)	anti-Nuc Total Ab (Positive/Negative)	anti-Spike Total Ab (U/mL)	anti-Nuc Total Ab (Positive/Negative)
Recovered COVID-19 Patients	10	232 (5.9–815.0)	All samples positive	<0.4 (<0.4–0.4)	All samples negative
Vaccinated volunteers day 0[a]	10	NA	NA	<0.4 (<0.4)	All samples negative
Vaccinated volunteers day 2	10	NA	NA	<0.4 (<0.4)	All samples negative
Vaccinated volunteers day 7	10	NA	NA	<0.4 (<0.4)	All samples negative
Vaccinated volunteers day 14	10	NA	NA	<0.4 (<0.4)	All samples negative
Vaccinated volunteers day 30	10	NA	NA	<0.4 (<0.4)	All samples negative
Vaccinated volunteers day 56[b]	8	79.0 (46.6–110.1)	All samples negative	<0.4 (<0.4)	All samples negative
Vaccinated volunteers day 70	8	>2500 (>2500)	All samples negative	14.3 (2.9–40.0)[c]	All samples negative
Vaccinated volunteers day 86	11	>2500 (>2500)	All samples negative	11.2(1.9–32.7)[c]	All samples negative
Vaccinated volunteers days 216–233	25	1558 (434->2500)[d]	All samples negative	2.6 (<0.4–11.4)[d]	Not tested

NA, sample not collected. [a] Saliva collected immediately prior to first dose of BNT162b2 vaccine. [b] Matched serum and saliva samples collected immediately prior to second dose of BNT162b2 vaccine. [c] Matched values not significantly different. [d]Values were lower than earlier time points.

after 6 months [19], yet our seropositivity rate was 100% among vaccinated volunteers after 6 months. Salivary antibody testing may prove useful in this context, and targeted studies examining population level salivary antibody detection relative to infection rates, hospitalizations, and deaths should be considered.

There is no defined role of saliva matrix to detect exposure to viral infection or vaccination, although others have monitored infection rates using home-based saliva collections with mail transport [9]. Indeed, saliva is appealing versus other home collections such as dried blood spots that require additional laboratory infrastructure and patient risk. Because we investigated only Total Ab assays, interassay comparisons are required in salivary matrix including assays targeting specific immunoglobulin classes. Interassay comparisons are available in serum for commercial assays performed on automated platforms, commercially available ELISAs measured using a plate reader, lab developed ELISAs, and neutralization assays traceable to the Wuhan strain. Generally, automated platforms were 85–100% sensitive for previous infection and all had specificities of greater than 81%. Importantly, the anti-Spike Total Ab assay was 100% sensitive and specific, whereas a pseudovirus neutralization assay was only 41% sensitive in the same diverse sample set [20]. Broader validation studies are required to compare assay performance in saliva.

The absence of salivary antibody detection among recovered COVID-19 patients suggests further optimizations are required for these assays to reliably detect previous infection in saliva. Indeed, a multiplex magnetic microparticle assay to 10 distinct SARS-CoV-2 specific antigens detected IgG, IgA, and IgM antibodies in the saliva of COVID-19 patients; and depending on the target antigen, salivary IgG assays had sensitivities of 46–100% and specificities of 98–100% for COVID-19 after $\geq$ 14 days post-symptom onset [3]. It is plausible that patients experiencing a mild clinical course do not elicit a strong enough immune response to detect salivary antibodies using serum thresholds.

Study limitations include the small sample size and possible selection bias. Further consideration of more diverse populations as well as exposure to different vaccines are necessary. Our streamlined approach offers minimal sample manipulation and can easily be adopted by other clinical laboratories.

5. Conclusion

Salivary antibodies were detected by the Roche anti-Spike Total Ab assay only after two doses of vaccine for up to 6 months, and not among individuals with a history of COVID-19. These findings have implications for public health and COVID-19 immunology. Further research is required to profile salivary antibody kinetics and to determine whether salivary antibody detection can be useful to inform public health or patient care.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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