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Journal of Experimental Pathology Research Article

# BNT162b2 mRNA COVID-19 Vaccine Elicited Antibody Responses in COVID-19-naïve Subjects

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## Abstract

**Introduction:** Both the Roche and Abbott quantitative total/IgG spike antibody (S-Ab) assays are now traceable to the 1st WHO International Standard units (Binding antibody unit per mL [BAU/mL]). We performed this study to compare S-Ab to neutralizing antibody (N-Ab) responses up to 90 days post-vaccination in COVID-19 naïve subjects.

**Methods:** Between January to June 2021, seventy-three hospital staff from Changi General Hospital, Singapore, were tested for antibody levels pre-vaccination and 20-, 40-, 60- and 90-days post-vaccination. All subjects had no history of prior COVID-19 infection. Serum samples were assessed for total S-Ab (Roche Elecsys Anti-SARS-CoV-2 S), IgG S-Ab (Abbott quantitative IgG S-Ab), IgM S-Ab (Abbott qualitative IgM S-Ab), N-Ab (Snibe Maglumi) and total/IgG nucleocapsid antibodies (Roche/Abbott).

**Results:** Nucleocapsid antibodies were negative in all samples, corroborating their COVID-19 naïve status. All participants had high total/IgG S-Ab and N-Ab 20-days post-vaccination (Abbott IgG range 265-7764 BAU/mL, median 2412 BAU/mL; Roche total range 274-6127 BAU/mL, median 2146 BAU/mL; N-Ab range 0.51-15.7ug/mL, median 3.48ug/mL). All subjects experienced a significant decline in antibody levels by day 40 post-vaccination but antibodies appeared to plateau by day 60 and 90. Total/IgG S-Ab and N-Ab remained positive with high titers in all subjects throughout. In contrast, IgM displayed an increasing number of negative cases from day 20-90, with only 9.4% of subjects positive for IgM by day 90 (n=3/32). Younger patients had a significantly higher response than older patients. There was good agreement between N-Ab and S-Ab (total/IgG). (Total S-Ab $r = 0.80$, $p<0.001$, and IgG S-Ab $r = 0.85$, $p<0.001$).

**Conclusion:** Post-vaccination, Total/IgG S-Ab and N-Ab levels were high and correlated well. However, the IgM response was sub-optimal and did not correlate with N-Ab. Total/IgG S-Ab expressed as BAU/mL were comparable and may be used as a surrogate for N-Ab.

**Keywords:** Spike antibodies, Neutralizing antibodies, SARS-CoV-2, BNT162b2 mRNA vaccine, COVID-19, Immunoassays, WHO international standard units

**Abbreviations:** COVID-19: Coronavirus Disease 19; BNT162b2: Pfizer-BioNTech mRNA COVID-19 vaccine; ChAdOx1-S: AstraZeneca recombinant COVID-19 vaccine; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus type 2; S-Ab: Spike Antibody; BAU/mL: Binding Antibody unit per mL; N-Ab: Neutralizing Antibody; Nuc-Ab: Nucleocapsid Antibody; ECLIA: Electro-Chemiluminescent Immunoassay; CLIA: Chemiluminescent Immunoassay

## Introduction

Coronavirus disease 19 (COVID-19) mRNA vaccines have gained international acceptance and have been proven to be safe and effective [1,2]. In a study of 3,950 frontline healthcare workers with no previous laboratory documentation of COVID-19 [3], full vaccination decreased the infection rates from 1.38 per 1000 person-days to

0.04 infections per 1,000 person-days. An extensive case-control study of 156,930 patients [4] vaccinated with either the Pfizer-BioNTech mRNA vaccine (BNT162b2) or AstraZeneca recombinant vaccine (ChAdOx1-S) displayed a vaccine effectiveness of up to 70% 10-13 days post-vaccination. Recent evidence [5] has also shown that the vaccine is 89.5% effective against the B.1.1.7 variant of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), and 75.0% effective against the B.1.351 variant. Further studies have shown that a second dose of BNT162b2 was associated with the prevention of severe COVID-19 [4]. Both the Roche and Abbott quantitative total/IgG spike antibody (S-Ab) assays are now traceable to the 1st WHO International Standard units (Binding antibody unit per mL [BAU/mL]); Abbott Architect BAU/mL = 0.142 x AU/mL (Abbott user circular), and Roche total S-Ab BAU/mL = 0.97 x U/mL (Roche user circular), allowing interchangeable interpretation of results between assays. However, there is scant literature [6,7] detailing quantitative S-Ab results, reported in BAU/mL, compared to quantitative neutralizing antibody (N-Ab). It is noteworthy that seropositive subjects have enhanced antibody responses compared to seronegative subjects [8-10]. Furthermore, studies on the effectiveness of the BNT162b2 vaccine [2,3] do not report the inclusion of a large number of Asian subjects, and there is a paucity of information regarding the regional (South East Asia) responses to the new vaccine. Thus, we performed this study to compare S-Ab responses up to 90 days post-vaccination in COVID-19 naïve subjects in an Asian population, evidenced by negative nucleocapsid antibodies (Nuc-Ab) (both total and IgG) at all time points. This was an observational knowledge-generation study, whose rationale was purely to study the antibody responses of our population to the new vaccine for which no information was currently available.

## Methods

### Study participants

Between January to June 2021, sixty-five hospital staff from Changi General Hospital, Singapore, were tested for antibody levels pre-vaccination and 20-, 40-, 60- and 90-days post-vaccination. All subjects had no history of prior COVID-19 infection. Our study included 17 males and 48 females. Participants' ages ranged from 24-90 years old (mean age 41.5 ± 14.1 years). Because the number of subjects tested at different time points varied due to different vaccination schedules, the antibody responses of the entire group was also compared to 26 subjects with complete data for all time points.

### Materials and instrumentation

Serum samples from peripheral venous blood were collected using the BD Vacutainer collection system (REF 367336) and BD Vacutainer SST tubes (REF 367986). Serum was obtained post-centrifugation and stored at -70 degrees Celsius prior to analysis.

The Roche Elecsys Anti-SARS-CoV-2 S is a quantitative double-antigen sandwich electro-chemiluminescent immunoassay (ECLIA) for total S-Ab, run on the Roche Elecsys e801 auto-analyser, with a positive threshold ≥ 0.78 BAU/mL, upper limit 243 BAU/mL, dilution range up to 1:100, limit of detection 0.34 BAU/mL, reported precision of 2.9% and 1.4% at 0.47 and 178 BAU/mL; reported assay sensitivity of 98.8% and specificity of 99.98%. The Abbott quantitative IgG S-Ab was assessed on the Abbott Architect (measuring range 3.0-5680 BAU/mL, positive ≥ 7.1 BAU/mL, reported precision 4.9% and 5.1% at 6.8 and 5115 BAU/mL); limit of detection 1.0 BAU/mL, reported sensitivity 66-99% and specificity 99.6%). The Abbott Architect qualitative SARS-CoV-2 IgM S-Ab assay (positive cut-off index (COI) ≥ 1.0) is a chemiluminescent immunoassay (CLIA) whose performance has been previously reported [11]. The Snibe N-Ab assay is a fully automated competitive quantitative CLIA (Snibe Maglumi), where sample N-Ab competes with ACE2 antigen immobilized on a solid phase for binding labelled recombinant SARS-CoV-2 S Receptor Binding Domain antigen. After the addition of starter reagents to initiate the chemiluminescent reaction, the light signal is measured as relative light units that is inversely proportional to the sample N-Ab. It has a measuring range of 0.05-30ug/mL with ≥ 0.3ug/mL regarded as positive; the reported inter-assay precision is 1.27% and 1.01% at 0.079 and 21.192 ug/mL, limit of detection 0.045 ug/mL, sensitivity of 100% and specificity 100%. To exclude previous/asymptomatic COVID-19 infection, we utilized previously evaluated CLIA/ECLIA Nuc-Ab assays (Abbott IgG, positive COI ≥ 1.4; Roche total antibody, positive cut-off 1.0) [12,13].

### Statistical analysis

Data were presented in either mean ± standard deviation or median [inter-quartile range], as appropriate. No indeterminate or missing results were used. Antibody titers at different time points were compared using Mann-Whitney U testing (MedCalc Statistical Software version 20, MedCalc Software Ltd, Ostend, Belgium), with $p<0.05$ considered statistically significant. Regression analysis was also performed for results between Roche/Abbott S-Ab and N-Ab post-vaccination. Our institution's IRB deemed this work exempt as this was part of routine laboratory evaluation of new assays. In addition, our IRB exempts studies on seroprevalence surveys. However, informed consent was still obtained from all volunteers as they needed to provide serum samples on multiple occasions. Compliance with STARD guidelines is enclosed (see Supplementary Table 1).

## Results

### Proof of COVID-19 naivety

As pre-vaccinated/vaccinated subjects may contract or have previously contracted asymptomatic/subclinical COVID-19 pre- or post-vaccination and confound the antibody response, Nuc-Abs were assessed at each blood draw on both the Roche and Abbott Nuc-Ab automated chemiluminescent assays. All samples were Nuc-Ab negative on both assays at all time points, thus confirming the COVID-19 naïve status of all participants.

### Spike and neutralizing antibody responses

At 20 days after complete vaccination, ALL participants had high S-Ab (Abbott IgG range 265-7764 BAU/mL, median 2412 BAU/mL; Roche total range 274-6127 BAU/mL, median 2146 BAU/mL) and N-Ab (range 0.51-15.7ug/mL, median 3.48ug/mL). However, only 83% (48/58) were IgM S-Ab positive. All subjects experienced a significant decline in antibodies by day 40 post-vaccination (Table 1 and 2). While antibody titers continued to decline thereafter, they appear to plateau by day 60 and 90. However, total/IgG S-Ab and N-Ab remained positive with high titers in ALL subjects (Figure 1). IgM displayed a progressive decline in antibody levels from day 20 to 90, with only 9.4% of subjects positive for IgM by day 90 (n=3/32). The trend in antibodies was also similar in 26 study participants who had continuous measurements at all time points (Figure 2): all 26 subjects experienced a decline in total/IgG S-Ab and N-Ab from day 20 to 90 but remained positive even at day 90.

**Table 1:** Titers of neutralizing antibodies, total, IgG and IgM spike antibodies, in all subjects pre-vaccination, 20-, 40-, 60- and 90-days after receiving the second dose of the BNT162b2 mRNA COVID-19 vaccine.

| Test | N | Low | High | 25th percentile | 75th percentile | Median |
|---|---|---|---|---|---|---|
| **Total antibodies (Roche) (BAU/mL)** | | | | | | |
| **Pre-vaccination** | 65 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| **20 days** | 59 | 274 | 6127 | 1269 | 2904 | 2146 |
| **40 days** | 50 | 274 | 3633 | 899 | 2363 | 1639 |
| **60 days** | 41 | 253 | 4353 | 892 | 2314 | 1454 |
| **90 days** | 32 | 233 | 4318 | 594 | 1627 | 1069 |
| **IgG antibodies (Abbott) (BAU/mL)** | | | | | | |
| **Pre-vaccination** | 65 | 0.04 | 3.21 | 0.33 | 1.04 | 0.58 |
| **20 days** | 58 | 265 | 7764 | 1593 | 3764 | 2412 |
| **40 days** | 50 | 185 | 5963 | 893 | 2621 | 1547 |
| **60 days** | 42 | 259 | 2549 | 675 | 1612 | 1007 |
| **90 days** | 32 | 111 | 1482 | 283 | 777 | 528 |
| **IgM antibodies (Abbott) (COI)** | | | | | | |
| **Pre-vaccination** | 65 | 0.01 | 0.77 | 0.02 | 0.06 | 0.03 |
| **20 days** | 58 | 0.13 | 13.6 | 1.21 | 4.52 | 2.39 |
| **40 days** | 50 | 0.09 | 15.8 | 0.64 | 2.18 | 1.32 |
| **60 days** | 42 | 0.07 | 2.96 | 0.37 | 1.29 | 0.86 |
| **90 days** | 32 | 0.09 | 1.94 | 0.18 | 0.48 | 0.32 |
| **Neutralizing antibodies (Snibe) (ug/mL)** | | | | | | |
| **Pre-vaccination** | 65 | 0 | 0.05 | 0 | 0.02 | 0.01 |
| **20 days** | 58 | 0.51 | 15.7 | 2.32 | 5.14 | 3.48 |
| **40 days** | 49 | 0.39 | 30 | 2.05 | 3.51 | 2.73 |
| **60 days** | 42 | 0.74 | 6.93 | 1.87 | 3.57 | 2.38 |
| **90 days** | 29 | 0.45 | 7.14 | 1.14 | 2.7 | 1.75 |

**Table 2:** Mann-Whitney U comparison between neutralizing, total, IgG and IgM antibody titers between 20/40-days, 40/60-days and 60/90-days after complete vaccination.

| **20 to 40 days** | | | | | |
|---|---|---|---|---|---|
| | **20 days Median** | **40 days Median** | **Median Diff** | **95%CI** | **p** |
| **Total (BAU/mL)** | 2146 | 1639 | -494 | -111 to -942 | 0.02* |
| **IgG (BAU/mL)** | 2412 | 1547 | -829 | -367 to -1349 | 0.001* |
| **IgM (COI)** | 2.39 | 1.32 | -0.95 | -0.33 to -1.74 | 0.002* |
| **Neutralizing (ug/mL)** | 3.48 | 2.73 | -0.66 | -0.02 to -1.33 | 0.04* |
| **40 to 60 days** | | | | | |
| | **40 days Median** | **60 days Median** | **Median Diff** | **95%CI** | **P** |
| **Total (BAU/mL)** | 1639 | 1454 | -73.5 | -473 to 290 | 0.65 |
| **IgG (BAU/mL)** | 1547 | 1007 | -521 | -915 to -185 | 0.003* |
| **IgM (COI)** | 1.32 | 0.86 | -0.45 | -0.92 to -0.12 | 0.007* |
| **Neutralizing (ug/mL)** | 2.73 | 2.38 | -0.31 | -0.83 to 0.27 | 0.27 |
| **60 to 90 days** | | | | | |
| | **60 days Median** | **90 days Median** | **Median Diff** | **95%CI** | **P** |
| **Total (BAU/mL)** | 1454 | 1069 | -370 | -763 to 4 | 0.05 |
| **IgG (BAU/mL)** | 1007 | 528 | -468 | -706 to -226 | 0.0001* |
| **IgM (COI)** | 0.86 | 0.32 | -0.45 | -0.74 to -0.18 | 0.0003* |
| **Neutralizing (ug/mL)** | 2.38 | 1.75 | -0.66 | -1.24 to -0.08 | 0.03* |

* *indicates a significant difference*

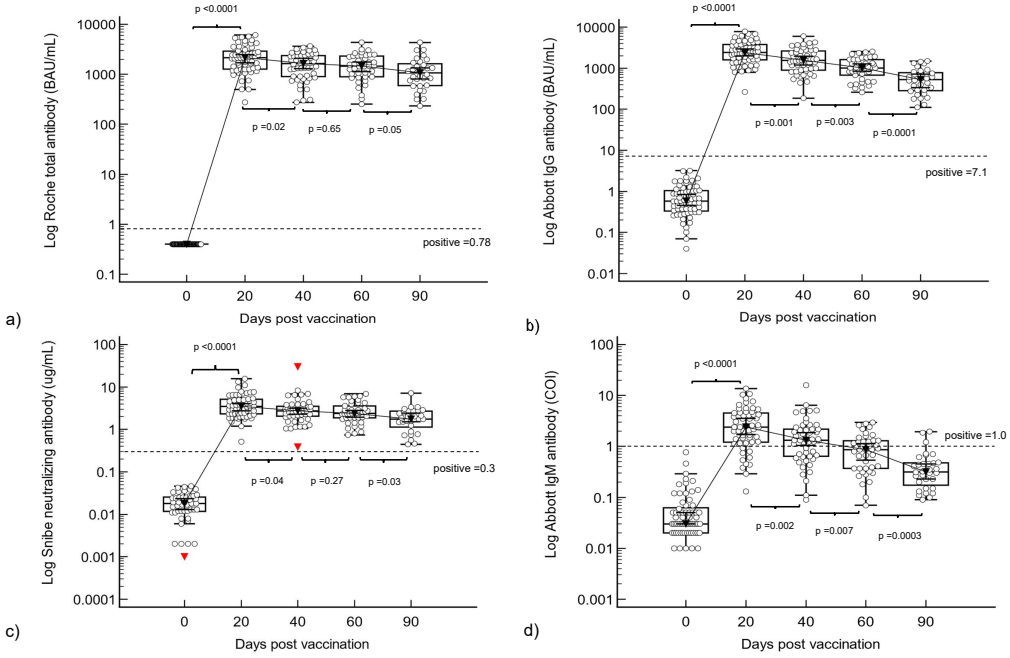


**Figure 1: a)** Roche total, **b)** Abbott IgG, **c)** neutralizing and **d)** Abbott IgM spike antibody responses in study participants at 0 days pre-vaccination (n=65), 20 days post-vaccination (Roche n=59, otherwise n=58), 40 days post-vaccination (Snibe n=49, otherwise n=50), 60 days post-vaccination (Roche n=41, otherwise n=42), and 90 days post-vaccination (Snibe n=29, otherwise n=32).

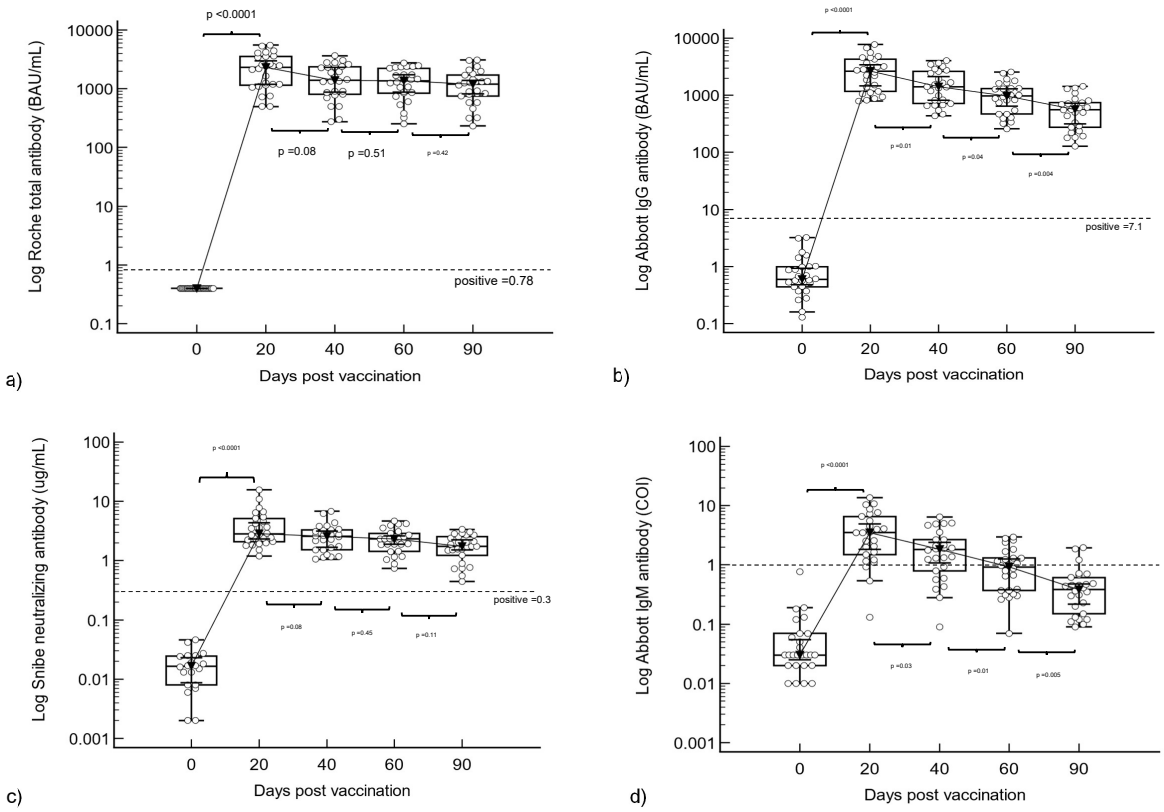


**Figure 2: a)** Roche total, **b)** Abbott IgG, **c)** neutralizing and **d)** Abbott IgM spike antibody responses in 26 study participants with antibody measurements at all time points (0 days pre-vaccination, 20, 40, 60 and 90 days post-vaccination).

### Gender and age analysis

Females had significantly higher IgG titers than males at 20- and 40-days post-vaccination, however, when all post-vaccination results were compared, Mann-Whitney U analysis showed no significant difference in antibody titers between genders (Table 3). Subjects <50 years old had significantly higher total/IgG/neutralizing antibody response than subjects ≥ 50 years old when all post-vaccination results were compared (Table 4).

**Table 3:** Mann-Whitney U comparison of all antibody titers between genders at 20-, 40-, 60-, 90-days after vaccination, as well as a Mann-Whitney U comparison of all male/female results after vaccination.

| Gender comparison | F | M | F Median | M Median | Median diff | 95% CI | p |
|---|---|---|---|---|---|---|---|
| **20 days** | | | | | | | |
| **Total (BAU/mL)** | 46 | 13 | 2351 | 1706 | -567 | -1386 to 74 | 0.09 |
| **IgG (BAU/mL)** | 46 | 12 | 2838 | 2061 | -959 | -2033 to -193 | 0.02* |
| **IgM (COI)** | 46 | 12 | 2.47 | 2.25 | 0.04 | -1.55 to 1.25 | 0.97 |
| **Neutralizing (ug/mL)** | 46 | 12 | 3.71 | 2.92 | -0.76 | -2.52 to 0.29 | 0.13 |
| **40 days** | | | | | | | |
| **Total (BAU/mL)** | 39 | 11 | 1747 | 1366 | -356 | -964 to 356 | 0.34 |
| **IgG (BAU/mL)** | 39 | 11 | 1655 | 1158 | -566 | -1276 to -2 | 0.048* |
| **IgM (COI)** | 39 | 11 | 1.34 | 1.18 | -0.02 | -0.78 to 0.9 | 0.98 |
| **Neutralizing (ug/mL)** | 38 | 11 | 3.07 | 2.39 | -0.62 | -1.49 to 0.25 | 0.16 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **60 days** | | | | | | | |
| **Total (BAU/mL)** | 34 | 7 | 1587 | 1144 | -414 | -1134 to 256 | 0.20 |
| **IgG (BAU/mL)** | 35 | 7 | 1043 | 675 | -405 | -896 to 51 | 0.06 |
| **IgM (COI)** | 35 | 7 | 0.91 | 0.41 | -0.12 | -0.72 to 0.75 | 0.62 |
| **Neutralizing (ug/mL)** | 35 | 7 | 2.51 | 2.04 | -0.65 | -1.93 to 0.14 | 0.14 |
| **90 days** | | | | | | | |
| **Total (BAU/mL)** | 27 | 5 | 989 | 1149 | 88 | -668 to 961 | 0.82 |
| **IgG (BAU/mL)** | 27 | 5 | 501 | 602 | 11 | -410 to 365 | 0.94 |
| **IgM (COI)** | 27 | 5 | 0.31 | 0.36 | 0.03 | -0.23 to 0.56 | 0.74 |
| **Neutralizing (ug/mL)** | 25 | 4 | 1.75 | 1.70 | -0.16 | NA | 0.61 |
| **All results after vaccination** | | | | | | | |
| **Total (BAU/mL)** | 146 | 36 | 1681 | 1543 | -244 | -599 to 90 | 0.14 |
| **IgG (BAU/mL)** | 147 | 35 | 1462 | 1022 | -351 | -729 to 3 | 0.052 |
| **IgM (COI)** | 147 | 35 | 1.16 | 1.32 | 0.14 | -0.24 to 0.56 | 0.43 |
| **Neutralizing (ug/mL)** | 144 | 34 | 2.73 | 2.40 | -0.46 | -1.02 to 0.04 | 0.07 |
| * *indicates a significant difference* | | | | | | | |

**Table 4:** Mann-Whitney U comparison of antibody titers between age groups at 20-, 40-, 60- and 90-days post-vaccination, as well as the Mann-Whitney U comparison of all results between age groups after vaccination.

| **Age comparison** | **≥ 50** | **<50** | **≥ 50 Median** | **<50 Median** | **Median diff** | **95% CI** | **p** |
|---|---|---|---|---|---|---|---|
| **20 days** | | | | | | | |
| **Total (BAU/mL)** | 15 | 42 | 1632 | 2261 | 313 | -360 to 1103 | 0.28 |
| **IgG (BAU/mL)** | 15 | 42 | 2186 | 2515 | 300 | -692 to 1176 | 0.51 |
| **IgM (COI)** | 15 | 42 | 2.55 | 2.36 | -0.26 | -1.8 to 1.05 | 0.70 |
| **Neutralizing (ug/mL)** | 15 | 42 | 2.71 | 3.65 | 0.69 | -0.36 to 1.90 | 0.20 |
| **40 days** | | | | | | | |
| **Total (BAU/mL)** | 16 | 34 | 1298 | 1785 | 225 | -390 to 845 | 0.49 |
| **IgG (BAU/mL)** | 16 | 34 | 1430 | 1588 | 115 | -569 to 751 | 0.77 |
| **IgM (COI)** | 16 | 34 | 1.08 | 1.32 | 0.3 | -0.42 to 0.82 | 0.45 |
| **Neutralizing (ug/mL)** | 16 | 33 | 2.60 | 3.12 | 0.48 | -0.39 to 1.31 | 0.25 |
| **60 days** | | | | | | | |
| **Total (BAU/mL)** | 13 | 28 | 1019 | 1645 | 426 | -119 to 958 | 0.08 |
| **IgG (BAU/mL)** | 13 | 29 | 892 | 1022 | 151 | -211 to 562 | 0.38 |
| **IgM (COI)** | 13 | 29 | 0.39 | 1.01 | 0.3 | -0.14 to 0.71 | 0.16 |
| **Neutralizing (ug/mL)** | 13 | 29 | 1.90 | 2.42 | 0.51 | -0.30 to 1.45 | 0.16 |
| **90 days** | | | | | | | |
| **Total (BAU/mL)** | 15 | 17 | 917 | 1249 | 135 | -372 to 690 | 0.69 |
| **IgG (BAU/mL)** | 15 | 17 | 395 | 602 | 92 | -215 to 361 | 0.64 |
| **IgM (COI)** | 15 | 17 | 0.23 | 0.44 | 0.16 | -0.01 to 0.32 | 0.06 |
| **Neutralizing (ug/mL)** | 11 | 18 | 1.57 | 1.95 | 0.18 | -0.66 to 1.02 | 0.65 |

| **All results after vaccination** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Total (BAU/mL)** | 59 | 121 | 1244 | 1776 | 405 | 107 to 717 | 0.01* |
| **IgG (BAU/mL)** | 59 | 122 | 1080 | 1498 | 324 | 5 to 621 | 0.046* |
| **IgM (COI)** | 59 | 122 | 0.8 | 1.23 | 0.26 | -0.01 to 0.6 | 0.06 |
| **Neutralizing (ug/mL)** | 55 | 122 | 2.24 | 2.84 | 0.56 | 0.14 to 1.03 | 0.01* |
| ** indicates a significant difference* | | | | | | | |

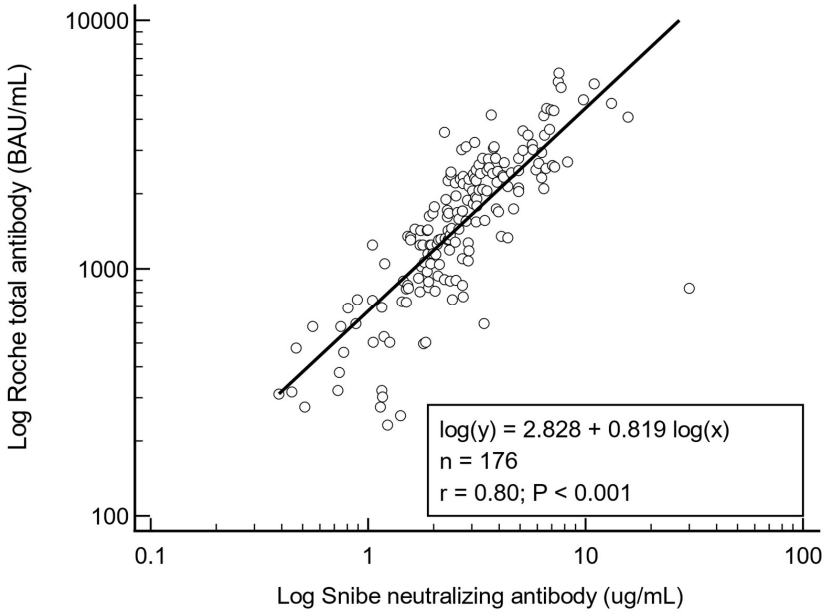


**Figure 3:** Correlation of all Log Roche total antibody and Log Snibe neutralizing antibodies after vaccination.

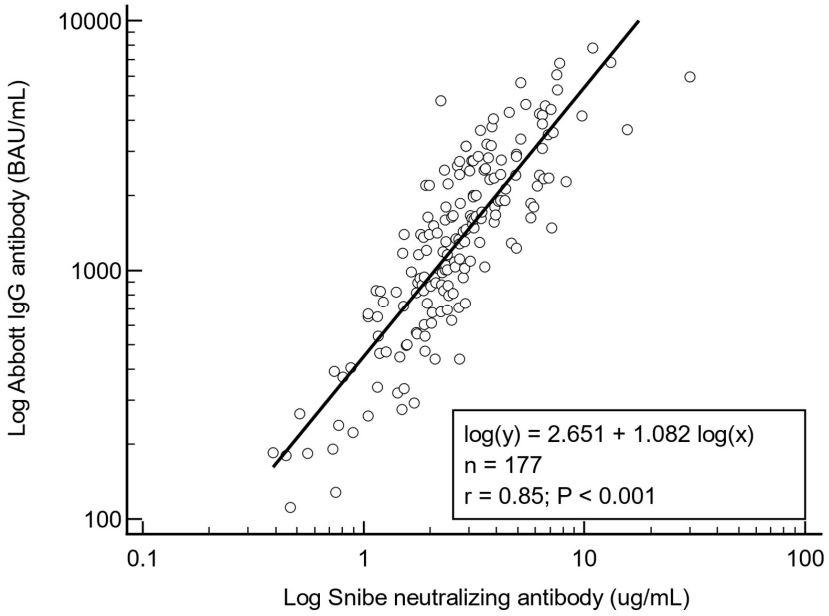


**Figure 4:** Correlation of all Log Abbott IgG antibody and Log Snibe neutralizing antibodies after vaccination.

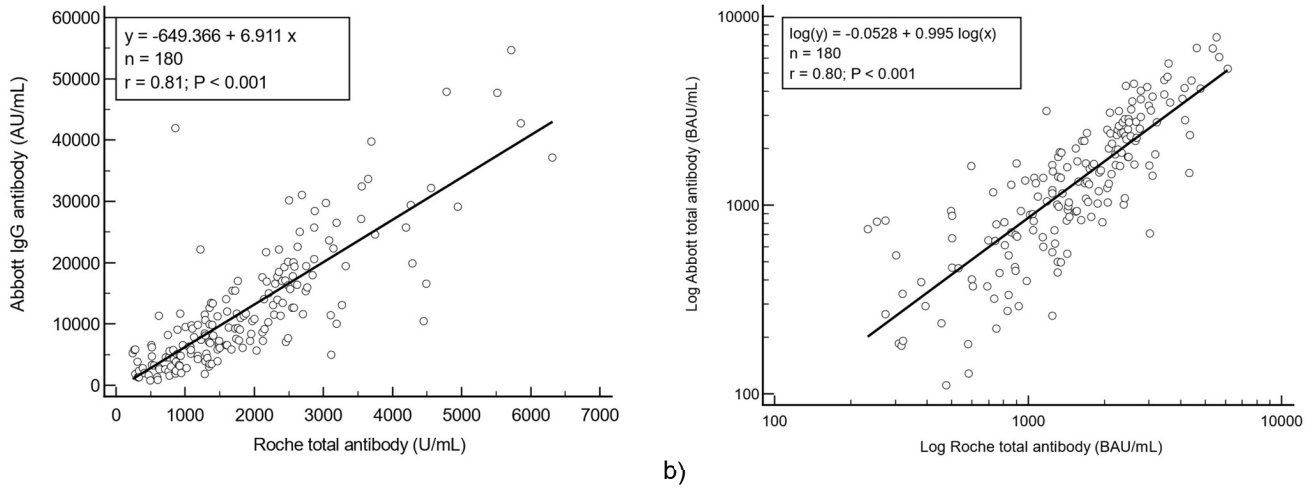


**Figure 5:** Correlation of all a) Abbott IgG antibody (AU/mL) and Roche total antibody (U/mL) and b) Log Abbott IgG antibody (BAU/mL) and Log Roche total antibody (BAU/mL) results after vaccination.

### Agreement of antibodies post-vaccination

After the second dose of vaccine, there was good agreement between N-Ab and S-Ab (total/IgG). Regression analysis showed: Log(Total S-Ab) = 0.819 log(N-Ab) + 2.828, r = 0.80, p<0.001, and Log(IgG S-Ab) = 1.082 log(N-Ab) + 2.651, r = 0.85, p<0.001 (Figure 3 and 4). However, the correlation of IgM and N-Ab post-vaccination was weaker than total/IgG S-Ab (r = 0.38, p<0.001).

We also compared the agreement of the Roche total S-Ab with the Abbott IgG S-Ab using manufacturer units (Roche in U/mL, Abbott in AU/mL) and in Log WHO units. While both had good agreement (r = 0.81 with manufacturer units, r =0.8 with WHO units), the slope and intercept were much improved using WHO units (slope 6.9 to 1.0, intercept -649 to -0.05) (Figure 5).

## Discussion

Nuc-Abs (total and IgG) remained negative in all subjects throughout the study, underscoring that our patients were COVID-19 naïve and free of any new infections post-vaccination. Expectedly, the BNT162b2 vaccine did not elicit any Nuc-Ab response. This was also demonstrated in other studies where initially seronegative subjects received the BNT162b2 vaccine [14,15]. Thus, Nuc-Ab responses remain useful for the assessment of COVID-19 breakthrough/re-infection in subjects vaccinated with BNT162b2.

Several studies have demonstrated that initially seronegative subjects mount a less robust antibody response compared to vaccinees with previous COVID-19 [8-10,16]. Serological testing should thus be reserved till after completing the second inoculation, when antibody titers are at their peak. At 20 days post-vaccination, all vaccinees had a brisk spike antibody response. Indeed, all samples exceeded the measuring range of the Roche total S-Ab assay and required sample dilution, while only 5 samples exceeded the upper limit of the IgG S-Ab assay. Both the IgG S-Ab and N-Ab assays are suitable for antibody assessment post-vaccination, but the Roche total S-Ab assay may require modification to accommodate such samples. Although the adequacy of post-vaccination antibody titers is not known at present, the observations in this study up to 90 days post-vaccination show elevated IgG S-Ab and N-Ab with values continuing to exceed the upper measuring range of the total S-Ab assay, indicating a very avid antibody response.

S-Ab and N-Ab titers were mostly unaffected by gender after complete vaccination in our study. However, younger patients (<50) demonstrated a greater antibody response compared to older subjects. Other studies [6,9] also show that lower N-Ab titers in older individuals after the BNT162b2 vaccine. While lower antibody titers in older subjects may give cause for concern as to vaccine efficacy in the elderly, the S-Ab titers remained quite high even in our older volunteers (> upper measuring range of the Roche assay). Indeed, larger studies of vaccinated adults in the real-world setting [17] have shown that there is only a slight difference in vaccine effectiveness between age groups: the BNT162b2 vaccine was 97.8% effective in preventing symptomatic COVID-19 at ages 16-44, 97.7% effective at ages 45-64, and 97.5% effective at ≥ 65 years old ≥ 14 days post-vaccination. Thus, even though antibody responses might differ slightly between age groups, it does not appear to be less effective in practice.

The fall in antibody titers post-vaccination has also been noted in another study (n=33) [18] using the mRNA Moderna vaccine. This antibody decline may be attributed to the acute T/B cells transitioning to memory T/B cells which can provide immunity for years to follow [19,20]. Our findings of the decline and subsequent stabilization of antibody titers after the second vaccination dose has also been seen in other studies [21] that have also observed a plateauing of total and IgG S-Ab titers 40 days post-vaccination. Indeed, other studies [22] have also demonstrated this decline of the seroprevalence of antibodies on a population level. The decline in antibody titers does not necessarily mean a loss of long-term immunity and remains to be investigated.

IgM S-Ab responses were underwhelming and less robust compared to IgG/total S-Ab, with only 15% of subjects IgM S-Ab reactive at 10 days after the first vaccine dose and 81% reactive 20 days post-vaccination. This has also been seen in the IgM response in COVID-19. In addition, IgM S-Ab did not correlate well with the N-Ab. We have previously noted that the role of IgM S-Ab was less useful compared to total and IgG Nuc-Ab responses in COVID-19 infections [11], except in the first week after RT-PCR positivity; with lower assay sensitivity at ≥ 14 days post-COVID-19 than even the IgG/total Nuc-Ab assays (IgM 77.8%, IgG Nuc-Ab 97.2%, total Nuc-Ab 97.2%). Other studies [23] have also shown that the sensitivity of IgM S-Ab or Nuc-Ab was less than that of IgG even by 4 weeks post-infection in patients with COVID-19 infection. There is good correlation between total/IgG S-Ab and N-Ab post-vaccination. Thus, IgG and total S-Ab, but not IgM S-Ab, may be used as a surrogate for the presence of neutralizing antibodies. Other studies [24] comparing antibody correlations also showed a good agreement between S-Ab (Euroimmun) assays to N-Ab (r = 0.874), with even stronger correlations in patients with severe COVID-19.

An additional finding was that when the units of the total and IgG S-Ab were converted to (Log) BAU/mL, the two assays achieved near equivalency as evidenced by the improved slope and intercept on regression analysis. This would lend support to the subsequent conversion of assessed values to BAU/mL, to allow standardization and comparison of results between the two assays.

We thus report the following novel findings:

- Ø Despite a slight decline, S-Ab and N-Ab levels remain positive at high titers up to 90 days post-vaccination, even in COVID-19 naïve subjects.
- Ø We report responses in BAU/mL, which allows for standardization and comparison of results between assays.
- Ø Younger subjects develop a higher antibody response to the vaccine.
- Ø Total and IgG S-Ab correlated well with the N-Ab and may be useful surrogates for of N-Ab.
- Ø IgM S-Ab performance was underwhelming, and did not correlate well with N-Ab.

A limitation of our study is that we did not explore the antibody responses to the first dose of the mRNA vaccine or antibody titers immediately prior to the second dose of the vaccine. This will assist in deciding upon the extension of the interval between vaccine doses. We also did not compare the antibody response of COVID-19 naïve subjects to patients with previous COVID-19. However, this has already been explored by others [8-10]. The antibody response to a single vaccine dose is quite effective, and individuals with previous infection typically mount a more vigorous antibody response. We were also unable to assess the antibody response to other vaccines. Our study is also in a single-center in a specific geographic area, and antibody responses may display differences between populations with different COVID-19 seroprevalences.

## Conclusion

In conclusion, Total/IgG S-Ab and N-Ab levels were high and correlated well post-vaccination despite a slight decrease in titers up to 90 days later. However, the IgM response was sub-optimal and did not correlate with N-Ab. Total/IgG S-Ab expressed as BAU/mL were comparable and may be used as a surrogate for N-Ab should the assay be unavailable. Expectedly, the antibody response was more robust in younger subjects.

## Disclosures

All co-authors have contributed to the study and manuscript.

## Conflicts of Interest

All authors report no conflicts of interest.

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