Research Article

Kinetics of immune responses to SARS-CoV-2 proteins in individuals with varying severity of infection and following a single dose of the AZD1222

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Abstract
To characterize the IgG and IgA responses to different SARS-CoV-2 proteins, we investigated the antibody responses to SARS-CoV-2 following natural infection and following a single dose of AZD1222 (Covishield), in Sri Lankan individuals. The IgG and IgA responses were assessed to S1, S2, RBD, and N proteins in patients at 4 weeks and 12 weeks since the onset of illness or following vaccination. Antibodies to the receptor-binding domain of SARS-CoV-2 wild type (WT), α, β, and λ and ACE2 (Angiotensin Converting Enzyme 2) receptor blocking antibodies were also assessed in these cohorts. For those with mild illness and in vaccines, the IgG responses to S1, S2, RBD, and N protein increased from 4 weeks to 12 weeks, while it remained unchanged in those with moderate/severe illness. In the vaccines, IgG antibodies to the S2 subunit had the highest significant rise (P < 0.0001). Vaccines had several-fold lower IgA antibodies to all the SARS-CoV-2 proteins tested than those with natural infection. At 12 weeks, the haemagglutination test (HAT) titres were significantly lower to the α in vaccines and significantly lower in those with mild illness and in vaccines to β and for λ. No such difference was seen in those with moderate/severe illness. Vaccines had significantly less IgA to SARS-CoV-2, but comparable IgG responses those with natural infection. However, following a single dose vaccines had reduced antibody levels to the VOCs, which further declined with time, suggesting the need to reduce the gap between the two doses, in countries experiencing outbreaks due to VOCs.

Keywords: immune responses, SARS-CoV-2 proteins, natural infection, AZD1222

Abbreviations: WT: wild type; HAT: haemagglutination test; VOC: variants of concern; RBD: receptor binding domain, sVNT: surrogate virus neutralization test.

Introduction
The COVID-19 pandemic due to the SARS-CoV-2 virus continues to cause significant mortality and morbidity and many countries are experiencing a worse situation than experienced at the beginning of the pandemic [1]. The emergence of SARS-CoV-2 variants of concern such as the B.1.1.7 (α) and more recently B.1.617.2 (λ) has led to the exponential increase in the number of COVID-19 cases and deaths in many countries [1–3]. While the higher income countries have vaccinated a large proportion of their population, resulting in lower case numbers, many lower income and lower-middle income countries are grappling with the increase in the case loads, overburdening of health care resources and the inability to secure adequate doses of COVID-19 vaccines [4].

Although the duration of protection against re-infection from SARS-CoV-2 is not known, it has been shown that re-infection does occur, especially among older individuals, probably due to waning of immunity [5]. Re-infection has shown to occur particularly with certain variants such as P.1 (γ) variant in Brazil despite a very high seroprevalence [6], and also with B.1.351
of Infectious Diseases (NIID), Sri Lanka, were recruited following informed written consent. They were followed throughout their illness while they were in hospital and clinical disease severity was classified as mild, moderate, and severe according to the WHO guidance of COVID-19 disease severity [22]. For this study, we recruited two cohorts of patients (Supplementary Table S1). Serum samples from the patient cohort 1 (n = 30) were used to determine the IgG and IgA antibody levels at 4 weeks since onset of illness, the ACE2 receptor-blocking antibody levels, and the antibodies to RBD by the HAT assay for the wild type (WT) and SARS-CoV-2 variants. The duration of illness was defined from the day or onset of symptoms and not the day of PCR positivity or admission to the hospital. Based on the WHO COVID-19 disease classification, 15 patients had mild illness and 15 patients had moderate/severe illness [22]. As all the patients in the first cohort could not be traced at 12 weeks, to carry out the above assays, we recruited a second cohort of patients. Based on the WHO COVID-19 disease classification, 14 patients had mild illness and 6 patients had moderate/severe illness [22].

To compare the antibody responses following infection with one dose of the AZD1222 vaccine, we recruited 20 individuals 4 weeks following vaccination and the same 20 individuals were followed at 12 weeks following vaccination. All 20 individuals who were included at 4 weeks following vaccination were included at 12 weeks following vaccination as well. We also included serum samples from individuals who had a febrile illness in 2017 and early 2018. Ethical approval was received by the Ethics Review Committee of Faculty of Medical Sciences, University of Sri Jayewardenepura. Informed written consent was obtained from patients.

**Luminex assay to measure SARS-CoV-2 S1, S2, RBD, and N specific IgA and IgG antibody responses**

SARS-CoV-2 S1, S2, RBD, and N specific IgA and IgG antibody responses were measured separately using multiplex SARS-CoV-2 antigen panels IgG and IgA (Millipore). The assay was carried out according to manufacturers instructions. The mean fluorescence intensity (MFI) was measured in each serum sample using MAGPIX® which was positively correlated with S1, S2, RBD, and N specific IgG and IgA in serum.

**Haemagglutination test (HAT) to detect antibodies to the receptor-binding domain (RBD)**

The HAT was carried out as previously described [23]. The B.1.1.7 (N501Y), B.1.351 (N501Y, E484K, K417N), and B.1.617.2 versions of the IH4-RBD reagent were produced as described [23], but included the relevant amino acid changes introduced by site-directed mutagenesis. These variants were titrated in a control HAT with the monoclonal antibody EY-6A (to a conserved class 4 epitope [23, 24]) and found to titrate identically with the original version so 100ng (50 μl of 2 μg/ml stock solution) was used for developing the HAT. The assays were carried out and interpreted as previously described [25]. The HAT titration was performed using 11 doubling dilutions of serum from 1:20 to 1:20,480, to determine the presence of RBD-specific antibodies. The RBD-specific antibody titre for the serum sample was defined by the last well in which the complete absence of “teardrop” formation was observed.

**Methods**

**Patients**

Patients confirmed SARS-CoV2 infection based on the positive RT-PCR who were admitted to the National Institute...
Surrogate neutralizing antibody test (sVNT) to detect NAbs

The surrogate virus neutralization test (sVNT) [26], which measures the percentage of inhibition of binding of the RBD of the S protein to recombinant ACE2 [26] (Genscript Biotech, USA) was carried out according to the manufacturer’s instructions as previously described by us [9]. Inhibition percentage ≥25% in a sample was considered as positive for NAbs.

Statistical analysis

Data were analysed by GraphPad Prism 9 version 9.2.0. The data were first tested for normality and homoscedasticity using Shapiro Wilk and Levene’s tests and since the assumptions were violated, non-parametric tests were used for the analysis. Kruskal–Wallis test was used to determine the difference between the antibody levels between the three different groups (two-tailed) followed by multiple comparisons using the two-stage step-up procedure of Benjamini, Krieger, and Yekutieli while controlling the false discovery rate (FDR) Mann–Whitney test (two-tailed) was used to determine the differences between antibody levels between 4 weeks and 12 weeks in those with natural infection. Wilcoxon paired t-tests (two-tailed) were used to determine the differences between antibody titres against S1, S2, RBD, N proteins, and ACE2 receptors in vaccinated individuals and the antibody titres to WT, B.1.1.7 (α), B.1.351(β), and B.1.617.2 (λ) between 4 weeks and 12 weeks in both naturally infected and vaccinated individuals. The antibody titres were compared between the WT, B.1.1.7 (α), B.1.351 (β), and B.1.617.2 (λ) at both time points for both naturally infected and vaccinated using the Friedman test followed by multiple comparisons using the two-stage step-up procedure of Benjamini, Krieger, and Yekutieli while controlling the false discovery rate (FDR).

Results

The kinetics of SARS-CoV-2 specific IgG responses in those with natural infection

IgG responses to the S1, S2, RBD, and N protein were measured in individuals with COVID-19 at 4 weeks and 12 weeks since the onset of illness and also in serum samples of 15 individuals who had a febrile illness in 2017 and early 2018. At 4 weeks since onset of illness, the highest magnitude of IgG antibody responses was seen for RBD in those with moderate/severe illness, whereas those with mild disease, had the highest responses to S2 (Fig. 1A, Table 1). Those who had a febrile illness in year 2017 and 2018 had significantly higher antibody titres compared to those with milder disease (Fig. 1B). At 12 weeks for all proteins, those with moderate/severe disease had significantly higher antibody levels than those with milder illness (Fig. 1B). The antibody responses only to N protein (P = 0.0137) was significantly different between the those with mild illness, moderate/severe disease and the vaccines as resulted by Kruskal–Wallis test (Fig. 1B). From 4 to 12 weeks, the S1 and RBD specific antibodies rose in those with mild illness, although they were not significant (Table 1). Patients who had moderate/severe illness sustained the same levels of antibodies for all four proteins from 4 weeks to 12 weeks. In the vaccines, from 4 weeks to 12 weeks the IgG levels to S1 (P = 0.0003), S2 (P < 0.0001), RBD (P = 0.0002) and N (P < 0.0001) had significantly increased (Table 1).

The kinetics of SARS-CoV-2 specific IgA responses in those with natural infection

IgA responses to the S1, S2, RBD, and N protein were measured in the above individuals with COVID-19 at 4 weeks and at 12 weeks since the onset of illness or following vaccination and also in serum samples of 15 individuals who had a febrile illness in 2017 and early 2018. At 4 weeks and 12 weeks of illness individuals with both mild and moderate/severe illness, had the highest levels of IgA antibodies to the RBD (Fig. 1C and D). However, those with moderate/severe disease had significantly higher antibody responses to all four proteins when compared to those with mild illness at 4 weeks, but there was no difference at 12 weeks (Table 1). Vaccines had similar responses to all four proteins, including the N protein at 4 weeks (Table 1). IgA levels for S1 (P = 0.004) and RBD (P = 0.0262) were significantly higher than the control group in the vaccines. However, at 4 weeks vaccines had significantly lower IgA levels to all proteins compared to those who had moderate/severe infection (Fig. 1C). Significant differences of IgA responses were seen in those with mild illness, moderate/severe illness, and vaccines for S1 (P = 0.001), S2 (P = 0.0003), RBD (P = 0.0003), and N protein (P = 0.04) at 4 weeks as resulted by Kruskal–Wallis test (Fig. 1C).

There was no difference in IgA levels to any of the proteins at 4 weeks compared to 12 weeks in patients with mild illness or with moderate/severe illness (Table 1). However, at 12 weeks, no significant differences were seen between the three groups to S1, S2, RBD, and N protein (Fig. 1D).

ACE2 receptor blocking antibodies following natural infection and one dose of AZD1222

Due to the lack of BSL-3 facilities to measure neutralizing antibodies, we used a surrogate test to measure the inhibition of binding of antibodies in patient sera to the ACE2 receptor [26]. This was shown to be 100% specific in the Sri Lankan population, with none of the sera of individuals collected in 2017 and 2018 giving a positive response [9]. The ACE2 blocking antibodies were significantly higher in those with moderate to severe illness when compared to those with mild illness at 4 weeks (P = 0.0306) and at 12 weeks (P = 0.0342) as reported previously (Fig. 2) [9]. However, in those
who received a single dose of the vaccine, the ACE2 blocking antibodies significantly reduced ($P < 0.0001$) from levels at 4 weeks (median 77.32, IQR 60.05–90.77% of inhibition) to 12 weeks (median 38.17, IQR 28.95–57.28% of inhibition).

Antibodies to the receptor binding domain of the spike protein, including variants, measured by the haemagglutination test (HAT)

HAT is a surrogate test to detect SARS-COV-2 NAbS, with high sensitivity and specificity, that correlate with neutralizing activity [27]. We previously evaluated the usefulness of the HAT assay in determining antibody responses to the RBD of the SARS-CoV-2, wild type (WT) virus, B.1.1.7 (α) variant, and the B.1.351 (β) variants at 4 weeks following a single dose of the AZD1222 vaccine and had also evaluated this assay in naturally infected individuals in Sri Lanka [28]. In this study, we proceeded to investigate the differences in the antibody responses to the RBD in those with natural infection at 4- and 12-weeks following infection, and after a single dose of the AZD1222 vaccine. The antibody responses to the WT, B.1.1.7 (α), B.1.351 (β), and B.1.617.2 (λ) were measured.

In those with mild illness, at 4 weeks from the onset of illness the median antibody titres to the WT was 160 (IQR 80–320), B.1.1.7 (α) was 120 (IQR 70–320), B.1.351 (β) was 10 (IQR, 0–80) and for B.1.617.2 (λ) it was 40 (IQR 20–80). The antibody titres for the WT was significantly higher compared to B.1.351 (β) ($P < 0.0001$) and B.1.617.2 (λ) ($P = 0.0004$) (Fig. 3A). At 12 weeks following the onset of illness, although there was a slight reduction in the antibody titres to the WT ($P = 0.44$) and B.1.617.2 (λ) ($P = 0.39$), this was not statistically significant (Table 2). In those with moderate/severe illness at 4 weeks from the onset of the illness the median antibody titres to the WT was 160 (IQR 80–320), B.1.1.7 (α) was 120 (IQR 70–320), B.1.351 (β) was 10 (IQR 0–80) and for B.1.617.2 (λ) it was 40 (IQR 20–80). The antibody titres for the WT was significantly higher compared to B.1.351 (β) ($P < 0.0001$) and B.1.617.2 (λ) ($P = 0.0004$) (Fig. 3A). At 12 weeks following the onset of illness, although there was a slight reduction in the antibody titres to the WT ($P = 0.44$) and B.1.617.2 (λ) ($P = 0.39$), this was not statistically significant (Table 2).
Table 1. Antibody responses to S1, S2, RBD, and N protein of the SARSCoV-2 in those with varying severity of illness and in those following a single dose of the AZD1222. MFU indicates the median fluorescence intensity.

|                           | 4 weeks Median (IQR) | 12 weeks Median (IQR) | P value |
|---------------------------|----------------------|-----------------------|--------|
| **Mild infection (IgG)**  |                      |                       |        |
| S1                        | 734 (483–1071)       | 1336 (24–4714)        | 0.59   |
| S2                        | 3503 (1656–5795)     | 3579 (106.8–9912)     | 0.68   |
| RBD                       | 539 (840–2960)       | 2952 (38.7–7516)      | 0.59   |
| N                         | 2094 (1554–4787)     | 2694 (51–7547)        | 0.84   |
| **Mild infection (IgA)**  |                      |                       |        |
| S1                        | 152 (79–490)         | 192 (19–422.1)        | 0.69   |
| S2                        | 354 (219–561.5)      | 380.2 (165.6–869)     | 0.71   |
| RBD                       | 656.5 (303–1616)     | 770.5 (180.3–1520)    | 0.98   |
| N                         | 207.5 (78–468)       | 276.3 (165.5–496.5)   | 0.31   |
| **Moderate/severe infection (IgG)** |                |                       |        |
| S1                        | 4776 (1395–7833)     | 5064 (2744–6038)      | 0.96   |
| S2                        | 6869 (2001–11 131)   | 8931 (7262–9607)      | 0.85   |
| RBD                       | 7486 (2784–10 218)   | 7829 (5083–8533)      | 0.67   |
| N                         | 5831 (3123–9383)     | 9538 (8810–10 844)    | 0.31   |
| **Moderate/severe infection (IgA)** |                |                       |        |
| S1                        | 1043 (220–1784)      | 391.8 (132.8–2021)    | 0.52   |
| S2                        | 934 (399–3679)       | 1378 (153.9–2269)     | 0.73   |
| RBD                       | 3375 (1192–5401)     | 4806 (165.6–869)      | 0.71   |
| N                         | 661 (211.5–6165)     | 273 (75.9–596.1)      | 0.18   |
| **Vaccinated IgG**        |                      |                       |        |
| S1                        | 2215 (1223–3870)     | 3969 (2805–6199)      | 0.0003 |
| S2                        | 1625 (1063–4329)     | 6537 (4570–12 690)    | <0.0001|
| RBD                       | 4393 (2355–6131)     | 9838 (4817–10 421)    | 0.0002 |
| N                         | 95 (57–591)          | 1482 (290–2447)       | <0.0001|
| **Vaccinated IgA**        |                      |                       |        |
| S1                        | 76.5 (38.2–166.5)    | 140 (25–921)          | 0.363  |
| S2                        | 203.3 (101.3–310.9)  | 585 (194–1855)        | 0.0017 |
| RBD                       | 327.5 (183–612.8)    | 360 (119–1902)        | 0.956  |
| N                         | 182 (96–375)         | 127 (47–330)          | 0.622  |

Figure 2. ACE2 receptor blocking antibodies in patients with varying severity of illness and following a single dose of the AZD1222 vaccine. ACE receptor blocking antibodies were measured by the surrogate virus neutralizing test following natural infection at 4 weeks in those with mild illness (n = 15) and moderate/severe illness (n = 15) and at 12 weeks in those with moderate/severe illness (n = 6). Antibodies were also measured at 4 weeks (n = 20) and 12 weeks (n = 20) in vaccines following a single dose of AZD1222. The Kurskal–Wallis test was used to determine the difference between the antibody levels between the three different groups (two-tailed) followed by multiple comparisons using two-stage step-up procedure of Benjamini, Kheger, and Yekutieli while controlling the false discovery rate (FDR). The lines indicate the median and the interquartile range.

Discussion
In this study, we investigated the kinetics of IgG and IgA responses to S1, S2, RBD, and N protein, ACE2 receptor...
blocking antibodies and antibodies against SARS-CoV-2 variants, in individuals at 4 and 12 weeks following natural infection and in those who had a single dose of the AZD1222. Based on the Luminex assays for IgG and IgA levels to S1, S2, RBD, and N, IgG antibodies to these proteins following vaccination were increased significantly from 4 weeks to 12 weeks. In mild illness, although not significant, antibodies for S1 and RBD rose from 4 weeks to 12 weeks. In the vaccines, the most significant rise was seen for the S2 subunit, while in those with mild illness the rise was seen for IgG antibodies for the RBD. In those with moderate/severe illness while there was no change in the IgG responses from 4 to 12 weeks but the responses to the N protein had increased although this was not significant. Unexpectedly, the antibodies against N proteins were also increased from 4 to 12 weeks, possibly due to asymptomatic infection in some individuals after a single dose of the AZD1222 vaccine. Therefore, the kinetics of antibody responses to S1, S2, RBD, and N appear to vary based on the severity of the natural infection and also appeared to be different in vaccines. Interestingly, blood samples of those who had a febrile illness in 2017 and 2018 also gave IgG and IgA high responses to the S2 subunit, suggesting the presence of S2 subunit cross-reactive antibodies, in these donors as previously seen in other studies [13, 14]. Following a single dose of the AZD1222 vaccine, the antibodies against S2 appear to continue to rise from 4 to 12 weeks, possibly due to stimulation of pre-existing cross-reactive memory B cell responses to the S2 subunit [14].

Figure 3. Comparison of antibody titres to RBD of the SARS-CoV-2 using the HAT assay in those with varying severity of infection and in vaccines. Antibody titres were measured in individuals with mild illness to the WT, B.1.1.7 (α), B.1.351 (β), and B.1.617.2 (λ) at 4 weeks (n = 15) and 12 weeks (n = 14) since the onset of illness (A), in those with moderate/severe illness at 4 weeks (n = 15) and 12 weeks (n = 6) since onset of illness (B) and in those who received one dose of AZD1222 vaccine at 4 weeks (n = 20) and 12 weeks (n = 20) following the vaccine (C). The difference between antibody titres to WT, B.1.1.7 (α), B.1.351 (β), and B.1.617.2 (λ) was determined using the Wilcoxon paired t-test (two-tailed). The lines indicate the median and the interquartile range.
SARS-CoV-2 specific IgA antibodies have been shown to be generated during early illness and have the potent neutralizing ability [15]. IgA antibodies to the RBD have been shown to develop earlier than IgG and while some studies have shown that serum IgA does not associate with clinical disease severity [15], patients who developed the severe disease were shown to have higher levels of virus-specific IgA [29]. Serum IgA was shown to activate neutrophils, thereby leading to the production of increased levels of inflammatory mediators leading to disease pathogenesis [16]. We found that at 4 weeks of illness, those with moderate/severe illness had significantly higher serum IgA to S1, S2, RBD, and N compared to those with mild illness, but these high levels of IgA declined except for S2 protein and there were no differences between these two groups at 12 weeks since the onset of illness. Vaccines had several fold lower IgA antibodies to all the SARS-CoV-2 proteins tested than those with mild and moderate/severe illness at 4 weeks and 12 weeks. The importance of serum IgA in preventing re-infection is currently unknown and if those with lower IgA have reduced protection is currently unknown.

Although the IgG antibodies to S1, S2, and the RBD rose from 4 to 12 weeks in the vaccines, the ACE2 receptor-blocking antibodies, which were shown to correlate with neutralizing antibodies significantly decreased [26]. The HAT assay, which also measures antibodies to the RBD and has shown to correlate well with the ACE2 receptor blocking assay and with neutralizing antibodies [23, 28], also showed that the RBD binding antibodies decreased from 4 to 12 weeks in the vaccines. This suggests that although ACE2 receptor blocking antibodies are reduced, the antibodies that bound to the S2 region increase which neutralizes the SARS-COV2 through inhibition of fusion and uncoating of the virus.

### Table 2

|                     | 4 weeks Median (IQR) | 12 week Median (IQR) | P value |
|---------------------|---------------------|----------------------|---------|
| **Mild infection**  |                     |                      |         |
| WT                  | 160 (80–320)        | 120 (0–400)         | 0.4392  |
| B.1.1.7             | 120 (70–320)        | 120 (35–400)        | 0.9548  |
| B.1.351             | 10 (0–80)           | 30 (0–80)           | 0.5651  |
| B.1.617.2           | 40 (20–80)          | 30 (0–80)           | 0.3947  |
| **Moderate/severe infection** |               |                      |         |
| WT                  | 1280 (160–1280)     | 480 (70–800)        | 0.2151  |
| B.1.1.7             | 640 (160–1280)      | 480 (70–800)        | 0.4492  |
| B.1.351             | 40 (0–160)          | 90 (20–200)         | 0.4373  |
| B.1.617.2           | 320 (80–1280)       | 60 (20–560)         | 0.2622  |
| **Vaccinated**      |                     |                      |         |
| WT                  | 80 (40–280)         | 80 (0–80)           | 0.0018  |
| B.1.1.7             | 40 (25–160)         | 40 (0–140)          | 0.3687  |
| B.1.351             | 20 (0–70)           | 20 (0–20)           | 0.2593  |
| B.1.617.2           | 20 (0–70)           | 10 (0–40)           | 0.1406  |
Apart from assessing antibodies to the RBD to the wild type, we assessed the antibodies to three other VOCs, B.1.1.7 (α), B.1.351 (β), and B.1.617.2 (λ). At 4 weeks following vaccination, the vaccines had a significantly lower levels of antibodies to the RBD of WT, B.1.1.7 (α), and B.1.617.2 (λ) compared to severe illness. The antibody levels among vaccines were significantly lower for B.1.1.7 (α), B.1.351 (β), and 1.617.2 (λ) compared to WT, showing a reduction in antibody binding to the RBD of the VOCs. These levels further declined at 12 weeks following vaccination, to VOCS, showing that a single dose of the AZD1222 was likely to offer less protection against VOCs. In fact, it has been shown that one dose of AZD1222 is only 33% effective in preventing symptomatic disease with B.1.617.2 (λ), 3 weeks following the first dose [30]. The efficacy of a single dose against B.1.617.2 (λ) is likely to decline further by 12 weeks, as the antibodies to RBD further waned. However, the efficacy of two doses of AZD1222 against hospitalization was 92%, while for Pfizer-BioNTech was 96% [31]. Therefore, in countries that have outbreaks due to VOCs, especially B.1.617.2 (λ), it would be prudent to reduce the gap between the two doses to increase efficacy as currently carried out in many countries. Interestingly, although those with mild or moderate/severe illness also had a marked reduction in antibodies to the RBD of B.1.351 (β), they had higher levels of antibodies to the RBD of B.1.617.2 (λ) at 4 weeks compared to B.1.351 (β). However, by 12 weeks the antibody levels to both B.1.351 (β) and B.1.617.2 (λ) were similar. Therefore, B.1.617.2 (λ) had less immune evasion than B.1.351 (β) in those who were naturally infected, at least during early convalescence.

In summary, we have investigated the kinetics and differences in IgG and IgA antibody responses to the S1, S2, RBD, and N in those with varying severity of infection and vaccines who received a single dose of AZD1222, which showed that vaccines had significantly less IgA to SARS-CoV-2, but comparable IgG responses those with natural infection. However, following a single dose vaccines had reduced antibody levels to the VOCs, which further declined with time, suggesting the need to reduce the gap between the two doses, in countries experiencing outbreaks due to VOCs.

**Supplementary data**

Supplementary data is available at Clinical and Experimental Immunology online.

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**Conflict of interest**

None of the authors have any conflicts of interest.

**Author contributions**

GNM, CJ and DJ: study design. CJ and DG: project administration. DG, CJ, HK, ISA, SD, BG, AW and RW: recruitment of study participants. DJ, LG, TTPJ, AK, PDP, TR, ISA, SD, BG and HK: carrying out experiments. LS and TKT: laboratory assay development and validation. GS, DJ and GNM: data analysis. GM and DJ: writing the manuscript. GNM, GSO, and AT: editing and proofreading the manuscript. CJ, GSO, AT, and GM: funding acquisition. All authors contributed to the article and approved the submitted version.

**Data availability statement**

All data are available within the manuscript, figures and the tables. Individual data points are shown in all figures. Source data are provided with this paper.

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