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RESEARCH ARTICLE

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Comparison of nucleocapsid and spike antibody ELISAs for determining SARS-CoV-2 seropositivity in Kenyan women and infants

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Abstract

A multitude of enzyme-linked immunosorbent assays (ELISAs) has been developed to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies since the coronavirus disease 2019 pandemic started in late 2019. Assessing the reliability of these assays in diverse global populations is critical. This study compares the use of the commercially available Platelia Total Ab Assay (Bio-Rad) nucleocapsid ELISA to the widely used Mount Sinai spike IgG ELISA in a Kenyan population seroprevalence study. Using longitudinal plasma specimens collected from a mother–infant cohort living in Nairobi, Kenya between May 2019 and December 2020, this study demonstrates that the two assays have a high qualitative agreement (92.7%) and strong correlation of antibody levels (R^2 = 0.973) in repeated

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measures. Within this cohort, seroprevalence detected by either ELISA closely resembled previously published seroprevalence estimates for Kenya during the sampling period and no significant difference in the incidence of SARS-CoV-2 antibody detection by either assay was observed. Assay comparability was not affected by HIV exposure status. These data support the use of the Platelia SARS-CoV-2 Total Ab ELISA as a suitable high-throughput method for seroprevalence studies in Kenya.

KEYWORDS
ELISA, Kenya, nucleocapsid, SARS-CoV-2, serology, spike

1 | INTRODUCTION

Throughout the coronavirus disease 2019 (COVID-19) pandemic, differences in morbidity and mortality rates related to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection have been reported in different global populations.[1] Notably, populations in Africa have had lower morbidity and mortality rates compared to the burden of disease in Europe and the United States, despite evidence of high rates of infection in African countries such as Kenya.[2–4] Thus, accurate serological tests are essential tools for monitoring the epidemiology of SARS-CoV-2, particularly in populations with low symptomatic disease. However, the reliability of these serologic tests needs to be validated across diverse populations worldwide to accommodate differences among populations with respect to the severity of infection or prior related coronavirus infections that may affect test sensitivity or specificity. Detecting antibodies via enzyme-linked immunosorbent assay (ELISA) is a standard high-throughput method that can be performed in most immunological laboratories and clinics. Understanding the performance of such assays in diverse global cohorts is crucial to accurately estimate the seroprevalence of SARS-CoV-2 infection, reinfection, and seroreversion rates—especially in populations where testing and infection tracking resources are limited.[5]

A variety of commercially available and research-grade ELISAs measure antibodies targeting either of two immunodominant SARS-CoV-2 proteins: spike, which binds host receptors for viral entry, and nucleocapsid, which is required for viral replication.[6–8] Natural SARS-CoV-2 infection results in the development of both spike-specific and nucleocapsid-specific antibodies, and although some studies show similar kinetics for these antibody responses after natural infection, others find that nucleocapsid antibodies appear earlier and decay more rapidly than spike antibodies.[9–12] Heterogeneity in levels and specificity of spike and nucleocapsid antibodies from natural infection with SARS-CoV-2 could impact the sensitivity and specificity of serological assays in diverse populations.[7,13] Importantly, current vaccine-induced immunity is limited to spike antibodies; thus, detection of antibodies against nucleocapsid antigen permits specific detection of natural infections in vaccinated populations.

Nucleocapsid and spike-antigen ELISAs have been used to study SARS-CoV-2 seroprevalence among specific groups in Kenya, such as blood donors, truck drivers, healthcare workers, and antenatal clinic attendees.[14–17] The Mount Sinai ELISA, which has been shown to demonstrate high specificity and sensitivity to the receptor-binding domain (RBD) and spike antigens, received US FDA Emergency Use Authorization (EUA) in 2020 and has been independently validated for use in Kenyan populations.[17–19] However, it is not commercially available and is both resource- and time-intensive as it requires both a screen and confirmatory ELISA, thus limiting its applicability in resource-limited settings.[17] The Platelia SARS-CoV-2 Total Ab ELISA (Bio-Rad) received a EUA, targets nucleocapsid total (IgM, IgG, and IgA) antibodies, and is a single-step, commercially available, all-inclusive kit that is convenient for high-throughput sample testing. While several studies have compared the specificity and sensitivity of SARS-CoV-2 serological assays to the Mount Sinai ELISA[20–23]; no side-by-side comparison using the Platelia ELISA has been performed. This study aimed to assess the reliability of the commercially available Platelia SARS-CoV-2 Total Ab Assay to qualitatively assess SARS-CoV-2 serostatus in longitudinal samples collected between May 2019 and December 2020 from a SARS-CoV-2 vaccine-naïve mother–infant cohort based in Nairobi, Kenya.

2 | METHODS

2.1 | Study participants and sample collection

Participant plasma was collected for SARS-CoV-2 serology testing as part of the Linda Kizazi Study, a prospective cohort study of virome transmission from mother to infant within a densely populated urban area in Nairobi, Kenya.[24] Human subjects approvals for study procedures were obtained from the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee and the University of Washington and Fred Hutchinson Cancer Center Institutional Review Boards. All participants provided written informed consent for participation in the parent cohort study and additional informed consent was collected for SARS-CoV-2 testing.

Between December 2018 to March 2020, 211 pregnant women in their third trimester were recruited from Mathare North Health Centre in Nairobi; a subset of these women and their infants subsequently consented to SARS-CoV-2 testing. Women were eligible to enroll in the Linda Kizazi Study if aged 18–40 years, between 28 and 42 weeks' gestation, planning to breastfeed, and had received ≥6 months of ART if living with human immunodeficiency virus (LWHIV). Mothers with planned cesarean section, with serious medical conditions, or who were taking antimicrobial or immunosuppressive medication other than for HIV prophylaxis were not eligible for enrollment in the parent cohort.

Following delivery, mother–infant pairs had clinic visits at Week 6, Week 10, Month 6, and every 3 months thereafter through 2 years postpartum. At each visit, data about current and recent symptoms of illness, healthcare visits, diagnoses, medications, and immunizations were recorded, a physical examination was performed, and samples, including blood and stool, were collected.

2.2 | Platelia SARS-CoV-2 Total Ab ELISA

Detection of anti-SARS-CoV-2 nucleocapsid antibodies (IgM/IgA/IgG) was performed with mother and infant plasma samples collected between January 1, 2020 and December 1, 2020, using the Platelia SARS-CoV-2 Total Ab ELISA as described by the manufacturer (Bio-Rad). In addition, if a participant's first available sample in the testing window was seropositive, samples collected before January 1, 2020 were tested. The Platelia ELISA was granted US FDA EUA in October 2020 with reported specificity of 94.9% and a sensitivity of 97.4%.[8] Briefly, 15 µl of plasma samples and kit controls (positive, negative, and cutoff) were diluted 1:5 in the sample diluent, and then 75 µl conjugate solution was added to diluted samples. After mixing, 100 µl of diluted sample and conjugate solution were added to reaction microplates. The microplates were sealed and incubated at 37°C for 1 h. Following incubation, the plates were washed six times with 400 µl of wash buffer, and 200 µl development buffer was added to all wells immediately. Plates were left to develop for 30 min in the dark, then 100 µl of stopping solution was added, and plates were immediately read at $OD_{450\,nm}$ (reference filter $OD_{620\,nm}$). Specimen ratios for every sample were calculated by dividing sample $OD_{450\,nm}$ by the mean $OD_{450\,nm}$ values of the cutoff controls. Sample results were considered negative if the specimen ratio was <0.8, positive if ≥1.0, and equivocal if ≥0.8 and <1.0 as per the manufacturer's instructions. Samples with equivocal results were retested, and if the result of the repeat test was discrepant, the sample was retested; two concordant results of the test were used for the final interpretation of the equivocal results.

2.3 | Mount Sinai ELISA

All plasma tested using the Platelia ELISA was also tested using the Mount Sinai SARS-CoV-2 antibody ELISA, which requires two-steps: (1) a SARS-CoV-2 RBD antigen ELISA screen to define clear negative and potential positive samples and (2) a full-length spike antigen ELISA to confirm the results of potential-positive samples.[18,20] This protocol of the combined RBD screening and spike ELISAs closely follows the protocols used in Mount Sinai's Clinical Pathology Laboratory, which received US FDA EUA in April 2020 and reports 95% sensitivity and 100% specificity.[20,25,26] For the RBD ELISA screen, 2.5 µl of plasma was heat-inactivated at 56°C for 1 h and diluted 1:50 in the dilution buffer (phosphate-buffered saline [PBS], 1% w/v nonfat dry milk powder, 0.1% Tween-20) for use in the RBD screening assay. Immunlon 2HB plates (Thermo Fisher Scientific; 3455) were coated with His-tagged RBD (2 µg/ml) in PBS and incubated overnight at 4°C. Plates were washed four times with PBS-T solution (PBS, 0.1% Tween-20) and blocked with 200 µl blocking buffer (PBS-T, 3% w/v nonfat dry milk powder) for 1 h at room temperature. After removing the blocking buffer, 100 µl of diluted participant plasma was added to a well, in addition to four wells with CR3022 anti-SARS-CoV-2 RBD monoclonal antibodies (0.5 µg/ml) as positive controls[27,28] and four normal human serum (GeminiBio; 100-110) as negative control wells. CR3022 was expressed in Expi293F cells and purified by established protein A and size-exclusion chromatography methods.[20] Subsequent steps included: four washes with PBS-T, addition of 50 µl goat anti-human IgG-Fc horseradish peroxidase (HRP)-conjugated antibody (Sigma-Aldrich; A0170) diluted 1:3000 in the dilution buffer for 1 h at room temperature, four washes with PBS-T, addition of 100 µl of TMB/E HRP substrate solution (Thermo Fisher Scientific; 34029), incubation in the dark for 5 min at room temperature, and then the addition of 100 µl of 1 N sulfuric acid and immediate reading of $OD_{450\,nm}$. RBD-screen-positive samples had an $OD_{450\,nm}$ reading greater than the average reading of all negative controls on the plate plus 5 standard deviations (cutoff value).

All RBD-screen negative samples were recorded with a final result of SARS-CoV-2 seronegative. All RBD-screen-positive samples were further tested using a full-length spike antibody ELISA. Maxisorp Immuno 384-well plates (Thermo Fisher Scientific; 464718) were coated with 2 µg/ml full-length spike ECD-His recombinant protein (Sino Biological; 40589-V08B1). Plasma titrations were tested using five threefold dilutions starting at 1:100. Each plate had two negative control dilution series, two positive control dilution series starting at 1 µg/ml CR3022, and an additional control plasma from an individual who had received 250 µg SARS-CoV-2 mRNA vaccine (Moderna; mRNA-1273). Plates were washed four times and read at $OD_{450\,nm}$. After reading the plates, $OD_{450\,nm}$ values were used to plot a titration curve for the serial dilutions and calculate the area under the curve (AUC) using GraphPad Prism (version 9.0.0 [86]). Specimen ratios were calculated by dividing the sample AUC by the mean AUC of negative controls to normalize values across plates. The cutoff value for specimen ratios was the average of all negative control specimen ratios plus 5 standard deviations.

2.4 | Statistical analyses

Generalized estimating equation (GEE) models with an independent correlation structure were used to evaluate the association between antibody levels for the assays to allow for repeated measures. Kaplan–Meier survival analysis was used to estimate the incidence of SARS-CoV-2 antibody detection by either assay among women and infants. The beginning time at risk was set to January 1, 2020 for all mothers and for infants born before this date. For infants born after January 1, 2020, time at risk began at their date of birth. All participants' time at risk ended at the estimated time of SARS-CoV-2 infection, the date of the last negative serology test, or was censored on the last day in the sampling window, December 1, 2020. The time of infection is estimated as the midpoint between the participant's last negative serology result or January 1, 2020 (whichever occurred later) and their first seropositive result. A log-rank test was used to compare time to SARS-CoV-2 antibody detection between the Mount Sinai ELISA and the Platelia ELISA. Odds ratios (ORs) were determined using Fisher's exact test to compare the detection of SARS-CoV-2 antibodies between assays. All statistical analyses were completed using R version 4.0.0.

3 | RESULTS

3.1 | Participant characteristics

Of the 211 mother–infant pairs enrolled in the Linda Kizazi cohort, 104 mothers and 89 infants consented to SARS-CoV-2 testing and had samples available. The maternal median age at enrollment was 28 years (range: 18–40 years) and 51 mothers in this study (49%) were living with HIV. Of the 89 infants, 55% were male, the median age at sample collection was 9.5 months (range: 1.3–18.9 months), and 46% were HIV-exposed uninfected infants. As previously described, most participants who consented to SARS-CoV-2 testing reported no COVID-19-like symptoms proximal to the time of seroconversion, with one-fifth of participants reporting mild symptoms. No hospitalizations or deaths occurred from SARS-CoV-2 infection.[24]

3.2 | SARS-CoV-2 antibody detection using spike and nucleocapsid-based assays

Using the Platelia and two-step Mount Sinai ELISAs, a total of 352 plasma samples were assayed for SARS-CoV-2 antibodies; 200 samples from 104 mothers and 152 samples from 89 infants (median: 2; range: 1–6 samples per participant) collected between January 1, 2020 and December 1, 2020, and prepandemic samples for four participants for which the first sample tested were seropositive. Of the 352 samples tested, 69 (19.6%) were identified as seropositive by the Platelia nucleocapsid ELISA and 51 (14.5%) were seropositive by the two-step Mount Sinai spike ELISA. Comparing concordance across Platelia and Mount Sinai ELISAs for each sample resulted in a 92.7% qualitative agreement (κ: 0.740, 95% confidence interval [CI]: 0.64–0.83; Table 1). Of the sample results that were discordant (26/352), 22 were seropositive by the Platelia ELISA and negative by Mount Sinai ELISA, while four were negative by Platelia and positive by Mount Sinai.

Next, antibody levels from the Platelia nucleocapsid and confirmatory Mount Sinai full-length spike ELISAs were compared for 108 samples that were potentially positive by the RBD screen (see Section 2 for positivity criteria). A strong correlation ($R^2 = 0.973$) of antibody levels was observed using GEE models to accommodate for repeated sampling (Figure 1). Interestingly, in either scenario of result discordance, samples demonstrated a wide range of detectable antibody levels, so disagreement between assays cannot be attributed to antibody levels hovering near the respective assay's limit of detection (Figure 1).

3.3 | Comparison of ELISAs to determine SARS-CoV-2 seroprevalence in individuals

Among the combined 104 mothers and 89 infants, SARS-CoV-2 seroprevalence was 26.4% (51/193) by the Platelia ELISA and 21.8% (38/193) by the Mount Sinai ELISA (91.2% agreement, κ: 0.760, 95% CI: 0.69–0.83; Supporting Information: Table 1). Of the mothers included in this study, 37 of 104 were ever seropositive by Platelia ELISA and 28 by Mount Sinai ELISA. Both assays identified 14 of 89 (15.7%) infants to be ever

TABLE 1 Qualitative agreement between Mount Sinai and Platelia SARS-CoV-2 ELISAs

All samples ($N = 352$)	Mount Sinai ELISA					
Platelia ELISA	Positive	Negative	Total	Percent agreement	κ (95% confidence)	p Value
Positive	47 (13.4%)	22 (6.2%)	69 (19.6%)	Total agreement = 92.7%	0.740 (0.64–0.83)	<0.001
Negative	4 (1.1%)	279 (79.3%)	283 (80.4%)			
Total	51 (14.5%)	301 (85.5%)	352			

Abbreviations: 95% CI, 95% confidence Interval; ELISA, enzyme-linked immunosorbent assay; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

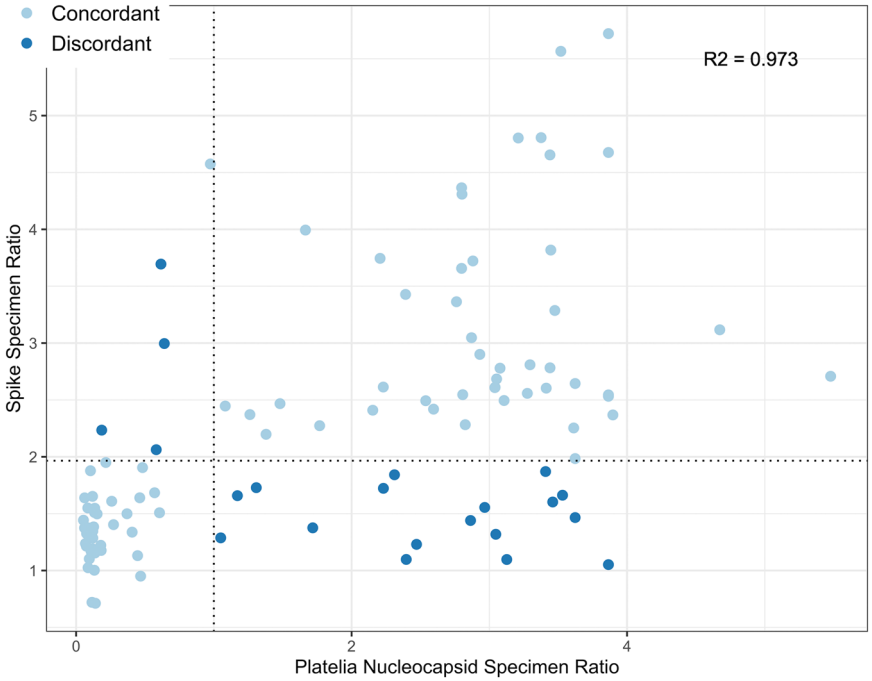

FIGURE 1 Correlation of anti-SARS-CoV-2 antibody levels by Mount Sinai spike confirmatory ELISA and Platelia ELISA. Samples with concordant Mount Sinai and Platelia ELISA results are shown in light blue ($N = 87$); samples with discordant results between ELISAs are in dark blue ($N = 21$). Specimen ratio positivity cutoffs for each respective ELISAs are denoted by the dotted line. All samples shown were tested by Platelia nucleocapsid–antigen ELISA, and then prescreened by Mount Sinai RBD ELISA before confirmatory testing using Mount Sinai full-length spike ELISA ($N = 108$). Samples with negative RBD-screening results were not shown. Correlation for repeated measures (R^2: 0973) calculated using GEE models. ELISA, enzyme-linked immunosorbent assay; GEE, generalized estimating equation; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

seropositive for antibodies against SARS-CoV-2, with the same 13 infants seropositive by both and two infants having positive results by one assay and negative results by the other. There was no significant variation in incidence of SARS-CoV-2 antibody detection by either assay for mothers ($p = 0.44$) or infants ($p = 0.98$) within the at-risk period (January 1, 2020 to December 1, 2020; Figure 2). Three mothers were positive for nucleocapsid antibodies by the Platelia ELISA before 2020 and were excluded from risk analyses. In all three cases, the pre-2020 samples positive by the Platelia ELISA were negative for SARS-CoV-2 antibodies against spike and RBD by the Mount Sinai ELISA. Additionally, two of the three mothers had later samples collected in mid-2020 that were positive for SARS-CoV-2 antibodies by both assays. There was no significant variation in detection of SARS-CoV-2 antibodies associated with maternal HIV status when tested by either Platelia (OR: 1.36, 95% CI: 0.57–3.31, $p = 0.54$) or Mount Sinai ELISAs (OR: 1.05, 95% CI: 0.40–2.75, $p = 1$; Supporting Information: Figure 1). Similarly, there was no significant increase in likelihood of antibody detection associated with infant HIV exposure by both Platelia ELISA (OR: 1.67, 95% CI: 0.46–6.63, $p = 0.40$) and Mount Sinai ELISA (OR: 1.20, 95% CI: 0.32–4.47, $p = 0.78$).

4 | DISCUSSION

This study demonstrates that the commercially available Platelia SARS-CoV-2 Total Ab Assay and two-step Mount Sinai SARS-CoV-2 RBD and spike IgG ELISA give comparable results when used on longitudinal samples collected from a cohort of unvaccinated Kenyan mothers and infants with mild or asymptomatic infection. For 352 plasma samples tested by both assays, a 92.7% qualitative agreement was observed with a strong correlation of antibody levels detected by Platelia nucleocapsid and Mount Sinai confirmatory spike ELISAs. These data resulted in a close agreement between seroprevalence measured by either Platelia ELISA (26.4%) or Mount Sinai ELISAs (21.8%). Similarly, comparing the incidence of SARS-CoV-2 antibody detection by either assay within the January 1–December 1, 2020 risk period showed no significant difference in both mothers and infants, with the results for infant participants being nearly identical. This study found no significant association between maternal HIV status or infant HIV exposure and SARS-CoV-2 antibody detection by either ELISA method. Finally, the SARS-CoV-2 incidence and seroprevalence rates measured in this cohort using either assay closely agree with previous reports of seroprevalence within urban communities in Kenya during the country's first two waves of SARS-CoV-2 infections.[3,17] Overall, these data support the use of the

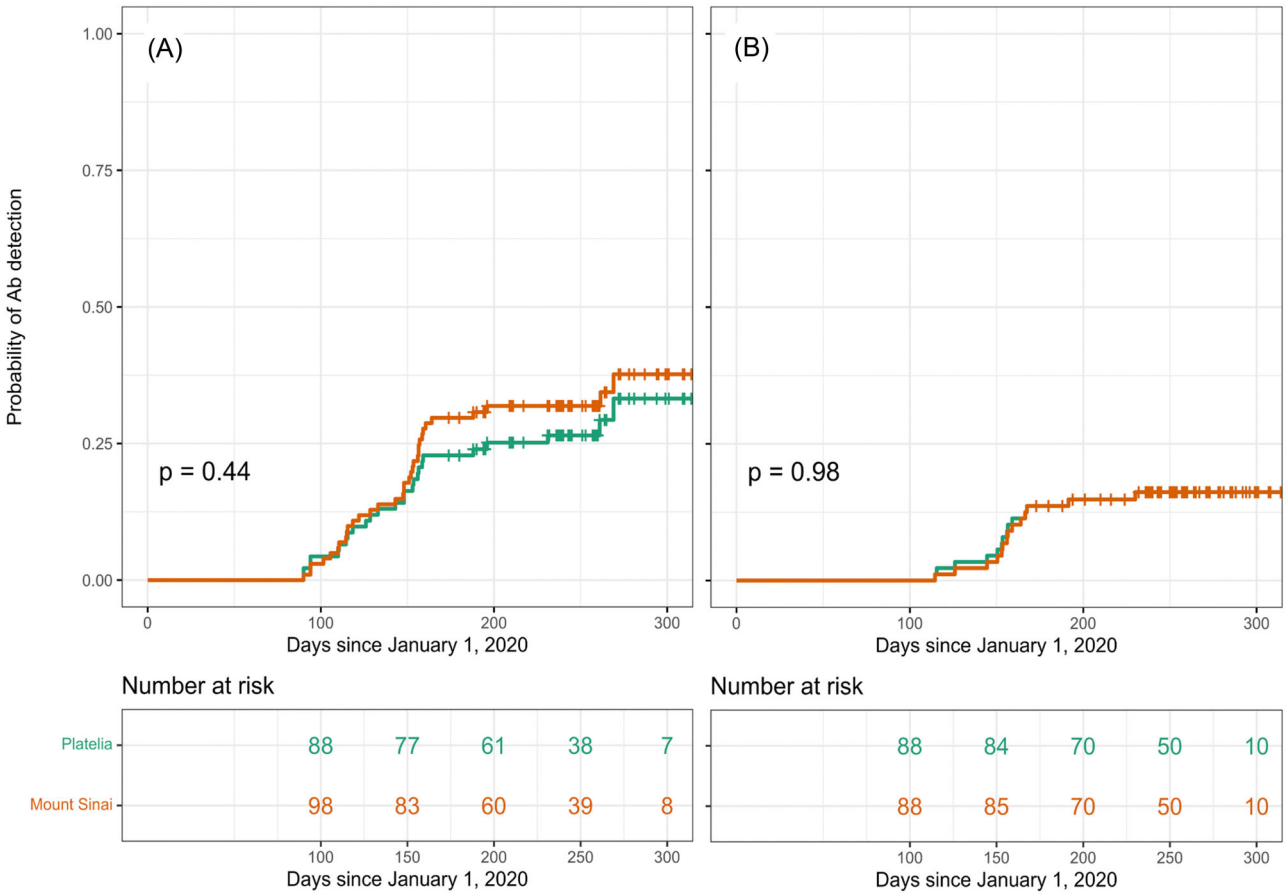

FIGURE 2 Antibody detection by Mount Sinai and Platelia ELISAs. Kaplan–Meier hazard functions for participants' estimated date of infection as detected by Platelia nucleocapsid ELISA (red) or Mount Sinai spike ELISA (green) for (A) all mother participants ($N = 104$) and (B) all infants ($N = 89$). The risk period is defined as January 1, 2020–December 1, 2020. Estimated time of infection is defined as the midpoint date between risk start or last seronegative result and first seropositive result. All participants entered the risk period on January 1, 2020, unless infants were born after this date, in which case they entered the risk period on their date of birth. Participants' time at risk ended at the estimated date of infection, the date of the last negative serology test, or was censored on December 1, 2020 (whichever occurred latest). Ab, antibody; ELISA, enzyme-linked immunosorbent assay; GEE, generalized estimating equation.

Platelia nucleocapsid ELISA for SARS-CoV-2 seroprevalence studies in Kenya.

As noted, the results of the Platelia and Mount Sinai ELISAs were not 100% concordant in the detection of SARS-Cov-2 antibodies. While it is possible that the discordant results may be attributed to differences in assay sensitivity and specificity, both assays are reported to demonstrate high sensitivity (Mount Sinai: 92.7%; Platelia 97.4%) and specificity (Mount Sinai: 99%; Platelia: 94.9%; Supporting Information: Table 2).[8,17] Notably, of the 26 (7.3%) qualitatively discordant samples in our study, there was a subset in which quantitative antibody levels were high by the Platelia nucleocapsid ELISA but low by the Mount Sinai spike ELISA, and vice versa (Figure 1). This quantitative discordance may be indicative of differences in the dynamics of the magnitude and durability of spike and nucleocapsid antibody responses over time after natural SARS-CoV-2 infection, as has been shown previously.[7,29,30] In addition, specimen volume used for each assay could contribute to differences observed, as the Platelia ELISA uses sixfold more plasma volume (15 µl) than the Mount Sinai ELISAs (≥2.5 µl; Supporting Information: Table 2). The detection of SARS-CoV-2 antibodies in pre-2020 samples from three women by the Platelia ELISA, but not the Mount Sinai ELISA, may suggest some infrequent cross-reactivity to nucleocapsid antigen of endemic coronaviruses within this adult population, as has been previously observed in studies of cross-reactive SARS-CoV-2 antibody responses.[31,32] The discrepancies we report are not unique, as other studies have shown that discrepancies in the results are likely to occur in participants with mild or asymptomatic infection,[24,30,32–34] which aptly describes this study's participants with only one-fifth of seropositive individuals having reported any symptoms of SARS-CoV-2 infection, all of which were mild.[24,30,32–34]

By utilizing a longitudinal, vaccine-naïve mother–infant cohort to assess the reliability of the Platelia ELISA, this study was able to survey the Platelia ELISA's efficacy in both women and their infants living in Kenya and determine its application to SARS-CoV-2 seroprevalence testing of longitudinal samples. Further, using the

Platelia ELISA for a population-based seroprevalence study highlighted the convenience of using a commercially available, one-step kit for reliable, high-throughput sample testing. Limitations of this study include the lack of confirmed SARS-CoV-2 infection in participants by viral RNA detection via nasal swabs. Because we do not have a set of verified SARS-CoV-2-positive and -negative samples, we are not able to evaluate the sensitivity and specificity of these serological assays from our data, and thus we examined reliability between the two assays, both of which are assumed to be imperfect. In addition, the 3-month sampling intervals utilized by this study limit the precise detection of SARS-CoV-2 infection and the ability to fully characterize subsequent antibody kinetics. A further limitation is that this study only compares assay efficacy in samples collected during the first year of the SARS-CoV-2 pandemic in Kenya using samples from a vaccine-naïve population. Routine validation of both SARS-CoV-2 antibody ELISAs in diverse global populations may be required to verify their sustained efficacy following the emergence of new viral variants and global vaccination efforts.

In summary, this study demonstrates that the Platelia SARS-CoV-2 Total Ab Assay and Mount Sinai SARS-CoV-2 RBD and spike IgG ELISAs perform comparably when used to identify SARS-CoV-2 antibodies in Kenya-based populations. The Platelia ELISA is an effective, high-throughput serological testing option for ongoing studies of SARS-CoV-2 seroprevalence in Kenya.

AUTHOR CONTRIBUTIONS

Prestone Owiti, Jennifer Slyker, Dara A. Lehman, and Bhavna H. Chohan conceived the study. Carolyn S. Fish, Prestone Owiti, Emily R. Begnel, Hannah L. Itell, Barbra A. Richardson, Soren Gantt, Efrem S. Lim, Jennifer Slyker, Julie Overbaugh, Dara A. Lehman, and Bhavna H. Chohan contributed to the design of the study. Prestone Owiti, Carolyn S. Fish, and Hannah L. Itell designed and performed experiments. Carolyn S. Fish, Prestone Owiti, Emily R. Begnel, Hannah L. Itell, Barbra A. Richardson, LaRinda A. Holland, Adam K. Khan, Rabia Maqsood, Efrem S. Lim, Jennifer Slyker, Dara A. Lehman, and Bhavna H. Chohan contributed to the data analysis. Emily R. Begnel, Ednah Ojee, Judith Adhiambo, Vincent Ogweno, Soren Gantt, Jennifer Slyker, John Kinuthia, Dalton Wamalwa, Dara A. Lehman, and Bhavna H. Chohan developed and managed the Linda Kizazi Cohort and contributed to the collection of participant samples and clinical data. Carolyn S. Fish, Prestone Owiti, Emily R. Begnel, Hannah L. Itell, LaRinda A. Holland, Barbra A. Richardson, Adam K. Khan, Rabia Maqsood, Soren Gantt, Efrem S. Lim, Jennifer Slyker, Julie Overbaugh, Dara A. Lehman, and Bhavna H. Chohan contributed to interpretation of the data. Carolyn S. Fish, Dara A. Lehman, and Bhavna H. Chohan wrote the first draft of the manuscript. All authors contributed to the writing and editing of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data sets generated and analyzed for this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Written informed consent was obtained from all participants or caregivers of children and additional written informed consent was obtained for SARS-CoV-2 testing. This study was approved by the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee, the University of Washington Institutional Review Board, and the Fred Hutchinson Cancer Center Institutional Review Board.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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