**Supplementary Materials**

*Antibody and malaria antigen multiplex testing*

For the pre-pandemic samples from 2018 NAIIS, the multiplex bead assay (MBA) for IgG against a panel of infectious and vaccine-preventable diseases was performed on the MAGPIX platform as described previously (1-3) with a serum dilution of approximately 1:400. The multiplex malaria antigen detection assay was also performed on the MAGPIX platform as described previously (4, 5) at a whole blood dilution of 1:40. All assays were performed at NRL. Malaria HRP2 antigen and antibody positivity for various *Plasmodium falciparum* antibodies (pfmsp1, pfama1, and glurp) was determined by using finite mixture models and defining a seropositive threshold for each as the mean plus two standard deviations from the distribution of the assumed seronegative population.

The Euroimmun NCP assay protocol consisted of sample plating, then incubation for 60 minutes at 37 °C. Two positive control wells, two negative control wells, and two calibrator control wells were included on each plate. A first wash step was done, followed by the addition of the enzyme horseradish peroxidase (HRP)-conjugated anti-human IgG, and then a second incubation for 30 minutes at room temperature (18-25°C). Wells were washed a second time, and a chromogen substrate solution was added. Following a third incubation at room temperature for 30 minutes, the reaction was stopped. After shaking the micro plate, the resultant absorbance was read on a microplate reader at 450 nanometer (nm) with reference at 650 nm.

Assay results are expressed as a ratio, calculated by dividing the ELISA optical densities (OD) of the sample by those of an internal calibrator provided with the test kit. A ratio <0.8 is considered negative, ≥0.8 to <1.1 borderline, and ≥1.1 positive. Borderline tests were repeated a second time and the second result taken as final.

The Abbott Architect CMIA assay results are expressed as the specimen result in relative light units from the chemiluminescent reaction divided by the average of three internal calibrator replicates; if the resulting ratio is <1.40, the specimen is considered negative, and if ≥1.40, positive. Any results deemed invalid by the analyzer were repeated a second time and the second result taken as final.

To determine the binding strength for IgG in cross-reactive samples, an avidity assay was conducted by introducing a urea wash step of various concentrations (2M and 8M, initially, then 4M and 5M) between sample incubation and detection antibody incubation on samples that were either borderline or positive, plus additional negative samples using the Euroimmun assay protocol. To determine the effect of the urea wash on true positive samples, it was also run on plasma collected from patients testing positive for SARS-CoV-2 at various time points post PCR confirmation. The urea wash step could not be used in the closed-system platform Abbott analyzer. By incubating with a denaturing agent, the urea wash would remove loosely-bound antibodies to the SARS-CoV-2 antigen target. The avidity assay for cross-reactive samples was performed by plating samples on a microplate and incubating for 60 minutes at 37 °C, then washing them. Diluted urea in phosphate buffered saline (PBS, 100μL) was added to all sample wells except control after the first Euroimmun wash. The plate was incubated for 10 minutes and washed prior to conducting the steps outlined above. This procedure was initially done twice, once with 2M urea and once with 8M urea concentrations, and then conducted with additional aliquots from the same samples with 4M and 5M urea wash at the NRL. An avidity index was calculated for each sample by the formula: (OD ratio to calibrator for urea exposed)/(OD ratio to calibrator for non-urea exposed) x 100%.

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2. Plucinski MM, Candrinho B, Chambe G, Muchanga J, Muguande O, Matsinhe G, Mathe G, Rogier E, Doyle T, Zulliger R, Colborn J, Saifodine A, Lammie P, Priest JW. 2018. Multiplex serology for impact evaluation of bed net distribution on burden of lymphatic filariasis and four species of human malaria in northern Mozambique. PLoS Negl Trop Dis 12:e0006278.
3. Priest JW, Jenks MH, Moss DM, Mao B, Buth S, Wannemuehler K, Soeung SC, Lucchi NW, Udhayakumar V, Gregory CJ, Huy R, Muth S, Lammie PJ. 2016. Integration of Multiplex Bead Assays for Parasitic Diseases into a National, Population-Based Serosurvey of Women 15-39 Years of Age in Cambodia. PLoS Negl Trop Dis 10:e0004699.
4. Plucinski MM, Herman C, Jones S, Dimbu R, Fortes F, Ljolje D, Lucchi N, Murphy SC, Smith NT, Cruz KR, Seilie AM, Halsey ES, Udhayakumar V, Aidoo M, Rogier E. 2019. Screening for Pfhrp2/3-Deleted Plasmodium falciparum, Non-falciparum, and Low-Density Malaria Infections by a Multiplex Antigen Assay. J Infect Dis 219:437-447.
5. Rogier E, Nace D, Ljolje D, Lucchi NW, Udhayakumar V, Aidoo M. 2020. Capture and Detection of Plasmodium vivax Lactate Dehydrogenase in a Bead-Based Multiplex Immunoassay. Am J Trop Med Hyg 102:1064-1067.

**Supplementary Tables and Figures**

Supplementary Table 1. Agreement between Euroimmun SARS-CoV-2 serological tests conducted at NIMR and at NRL for pre-pandemic samples (2018 NAIIS)

| | | NRL Euroimmun NCP results | | | |
|---|---|---|---|---|---|
| | | Borderline | Negative | Positive | Total |
| NIMR Euroimmun NCP results | Borderline | 2 | 2 | 3 | 7 |
| | Negative | 7 | 154 | 6 | 167 |
| | Positive | 4 | 7 | 27 | 38 |
| | Total | 13 | 163 | 36 | 212 |

Note: kappa = 0.6220 for all results; kappa = 0.7655 if borderline results excluded.

Supplementary Table 2. Agreement between Abbott SARS-CoV-2 serological tests conducted at NIMR and at NRL for pre-pandemic samples (2018 NAIIS)

| | | NRL Abbott results | | |
|---|---|---|---|---|
| | | Negative | Positive | Total |
| NIMR Abbott results | Negative | 196 | 3 | 199 |
| | Positive | 0 | 10 | 10 |
| | Total | 196 | 13 | 209 |

Note: kappa = 0.8621.

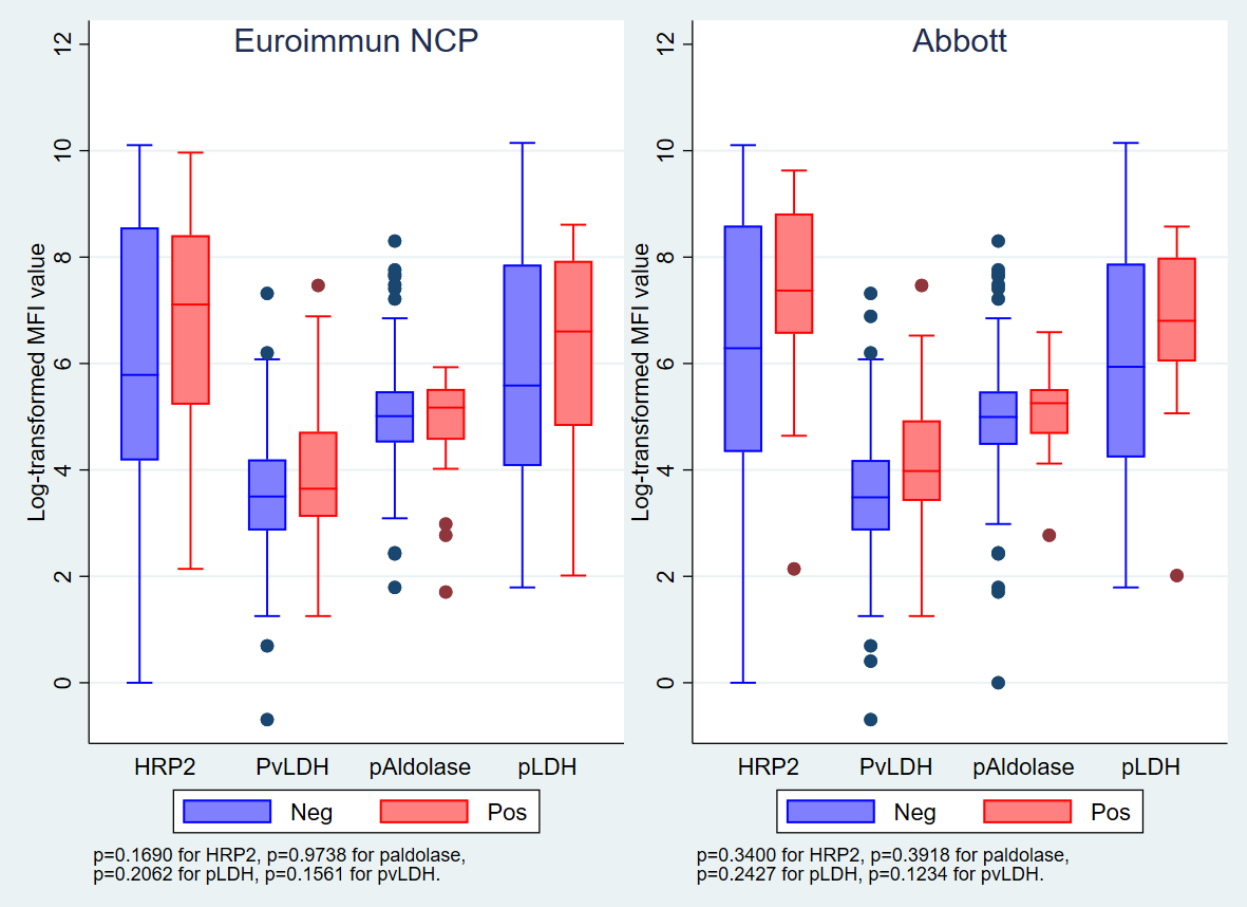
Supplementary Table 3. Relationship between antibody log values to a panel of other infectious diseases and false positivity on the Euroimmun and Abbott tests

| Disease | Pathogen | Antigen | p-value from Wilcoxon rank sum test for Euroimmun | Notes (when p-value significant) | p-value from Wilcoxon rank sum test for Abbott | Notes (when p-value significant) |
|---|---|---|---|---|---|---|
| Malaria (minimum panel of species-specific targets) | *Plasmodium falciparum* | Pf MSP1-19 | 0.0349 | | 0.4082 | |
| | | Hrp2 | 0.169 | | 0.3400 | |
| | | Glurp | 0.0119 | Ab values higher in positives | 0.3423 | |
| | | Csp | 0.0037 | Ab values higher in positives | 0.0056 | |
| | | Ama1 | 0.0112 | Ab values higher in positives | 0.0302 | |
| | | lsa | 0.1595 | | 0.1479 | |
| | *Plasmodium malariae* | Pm MSP1-19 | 0.0002 | Ab values higher in positives | 0.0026 | Ab values higher in positives |
| | *Plasmodium ovale* | Po MSP1-19 | 0.0059 | Ab values higher in positives | 0.0042 | Ab values higher in positives |
| | *Plasmodium vivax* | Pv MSP1-19 | 0.0766 | | 0.1333 | |
| Lymphatic filariasis | *Wuchereria bancrofti* | Wb123 | 0.1228 | | 0.0116 | Ab values higher in positives |
| | | Bm14 | 0.4666 | | 0.0337 | |
| | | Bm33 | 0.2483 | | 0.0327 | |
| Onchocerciasis | *Onchocerca volvulus* | OV-16 | 0.1235 | | 0.0041 | Ab values higher in positives |
| | | OV-33 | 0.0529 | | 0.0147 | Ab values higher in positives |
| Schistosomiasis | *Schistosoma spp.* | SEA | 0.476 | | 0.3681 | |
| Strongyloidiasis | *Strongyloides stercoralis* | NIE | 0.0443 | | 0.7326 | |

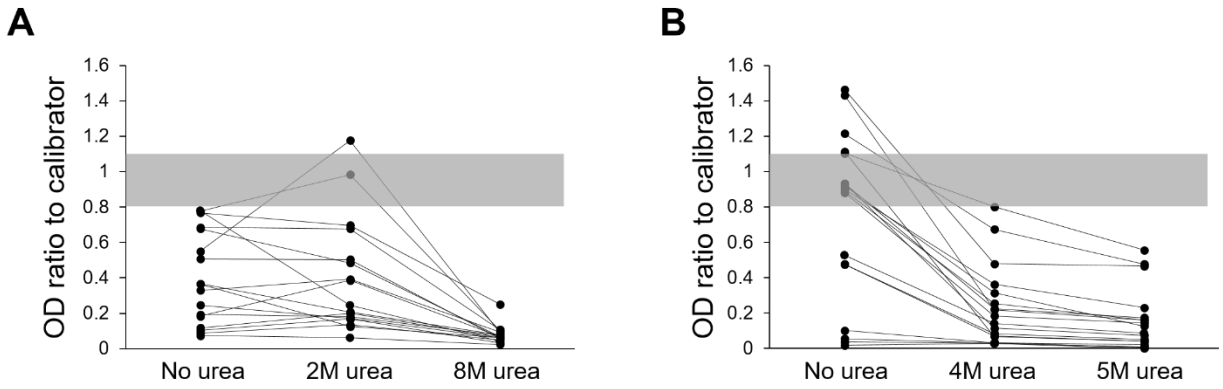
| | | | | | | |
|---|---|---|---|---|---|---|
| Trachoma | *Chlamydia trachoma* | Pgp3 | 0.0467 | | 0.0296 | |
| Syphilis/yaws | *Treponema pallidum* | r-p17 | 0.0815 | | 0.1291 | |
| | | TmpA | 0.0012 | Ab values higher in positives | 0.1602 | |
| Cysticercosis | *Taenia solium* | T24H | 0.5689 | | 0.0106 | Ab values higher in positives |
| Taeniasis | *Taenia solium* | rES33 | 0.1379 | | 0.0189 | Ab values higher in positives |
| Measles | Measles virus | Whole virus | 0.3724 | | 0.596 | |
| Rubella | Rubella virus | Whole virus | 0.7898 | | 0.6838 | |
| Diphtheria | *Corynebacterium diphtheria* | Diphtheria toxoid | 0.4613 | | 0.4082 | |
| Tetanus | *Clostridium tetani* | Tetanus toxoid | 0.5259 | | 0.5155 | |
| Campylobacteriosis (*C. jenuni)* | *Campylobacter jejuni* | campy 18 | 0.8957 | | 0.8746 | |
| | | campy 39 | 0.7689 | | 0.7937 | |
| Cholera | *Vibrio cholerae* | Cholera | 0.0007 | Ab values higher in negatives | 0.1076 | |
| ETEC infection | *Enterotoxigenic Escherichia coli I (ETEC)* | labile toxin β subunit | 0.0013 | Ab values higher in negatives | 0.9831 | |
| Cryptosporidiosis | *Cryptosporidium parvum* | Cp17 | 0.0727 | | 0.0637 | |
| | | Cp23 | 0.2166 | | 0.1139 | |
| Toxoplasmosis | *Toxoplasma gondii* | SAG2 | 0.0863 | | 0.1917 | |
| Giardiasis | *Giardia lamblia* | VSP3 | 0.2246 | | 0.6646 | |
| Salmonellosis | *Salmonella enterica serotype typhimurium* | SalB | 0.1824 | | 0.8987 | |
| | *Salmonella enterica serotype enteritidis* | SalD | 0.3756 | | 0.2944 | |

Note: grey shading indicates positive statistical significance after accounting for a false discovery rate of 10%.

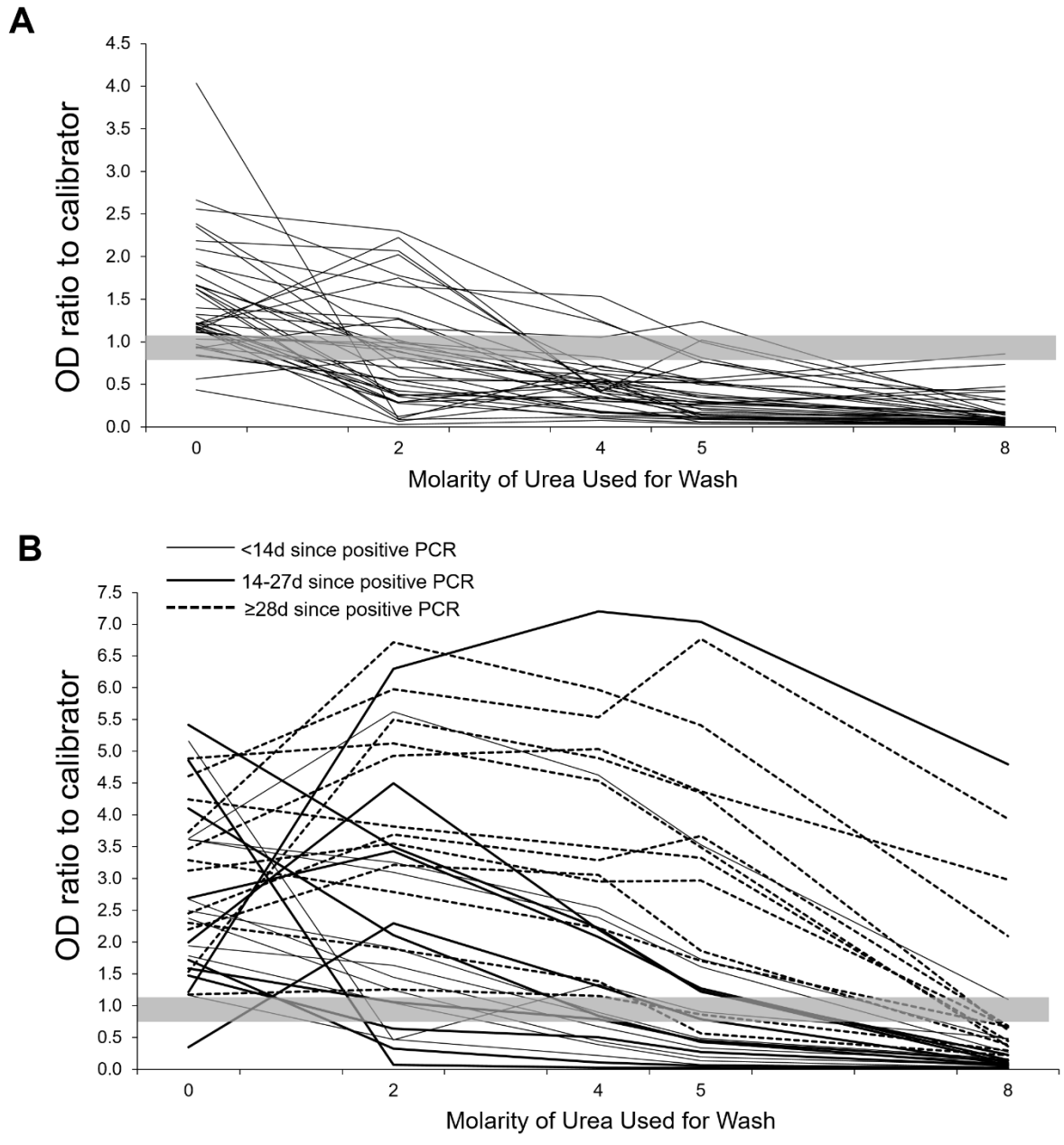
Supplementary Figure 1. Levels of *Plasmodium* antigens for pre-pandemic samples (2018 NAIIS) as determined by malaria antigen detection assay for: Euroimmun (n=168 for negative, n=38 for positive), and Abbott (n=197 negative, n=13 positive) SARS-CoV-2 antibody test result. Boxes shows interquartile range (IQR), lines displaying median, and whiskers extending 1.5x above and below IQR. Markers display values outside if 1.5x IQR.



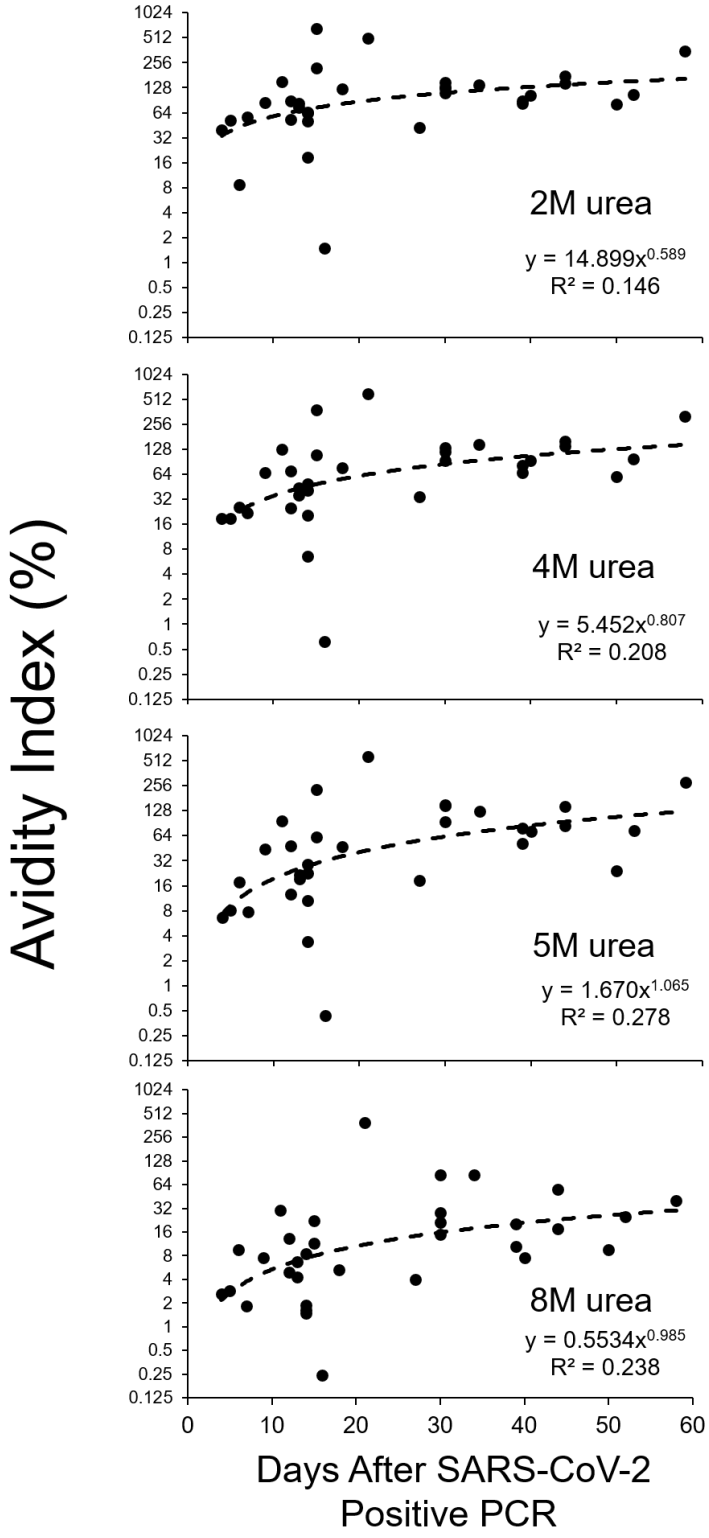
Supplemental Figure 2. Removal of weakly-bound IgG from pre-pandemic samples (2018 NAIIS) after incubation with protein denaturant urea for samples with inadequate volume to test for all four concentrations: 2, 4, 5, 8M urea. (A) The optical density (OD) to plate calibrator ratio for samples with 2M and 8M washes only (n=17). (B) The optical density (OD) to plate calibrator ratio for samples with 4M and 5M washes only (n=18). For both plots, the ratio range from 0.8 to 1.1 is highlighted grey and would elicit a 'borderline' call, with 'positive' samples above, and 'negative' samples below.



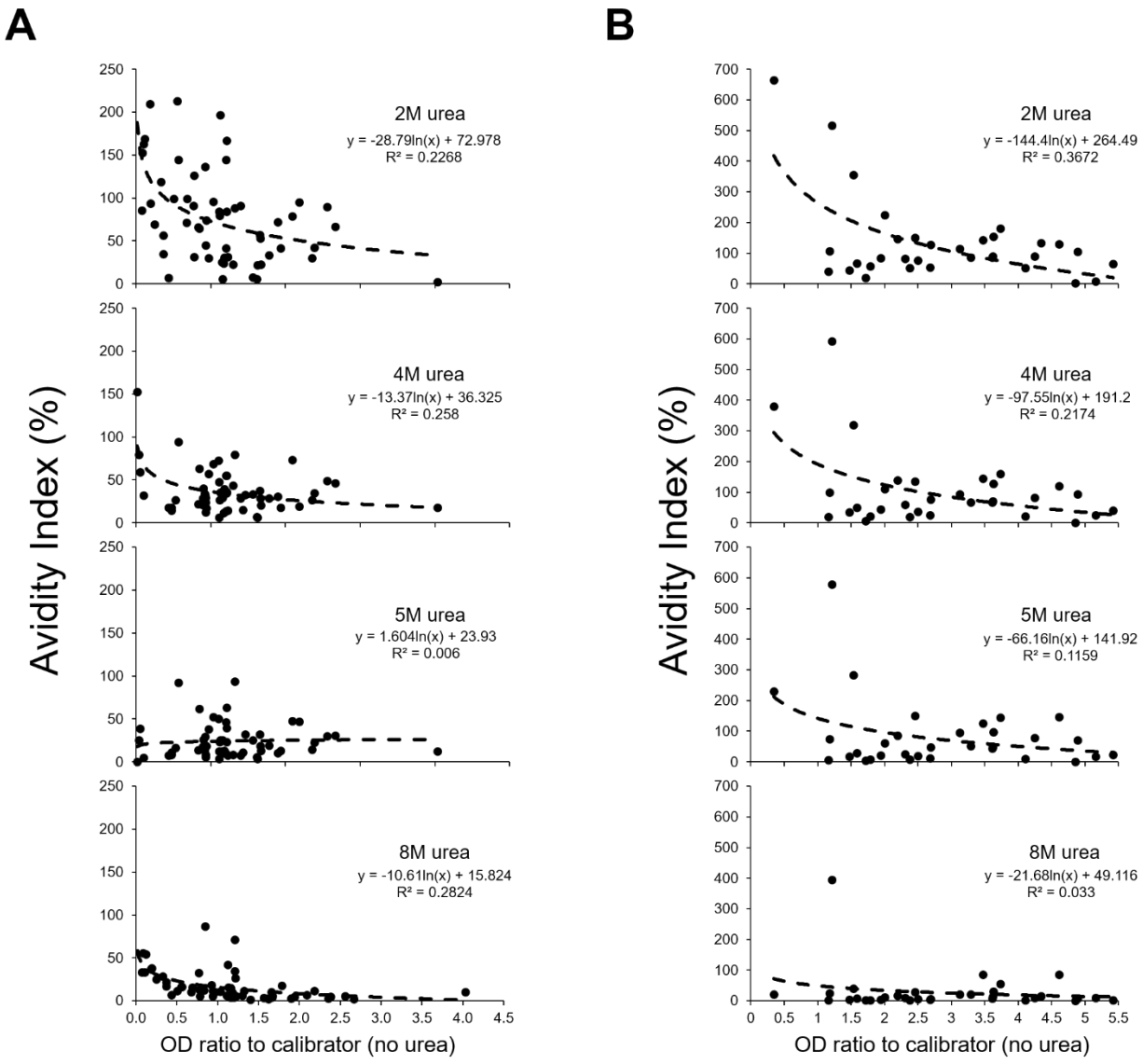
Supplemental Figure 3. The optical density (OD) to plate calibrator ratio for all individual samples with or without urea-based avidity assays at 2M, 4M, 5M, and 8M with the Euroimmun NCP assay for 40 pre-pandemic samples (2018 Nigeria HIV/AIDS Indicator and Impact Survey) (A) and for 32 samples from persons with previous SARS-CoV-2 positive PCR (B). For both (A) and (B), the optical density (OD) to plate calibrator ratio for all individual samples by molarity of urea wash. The ratio range from 0.8 to 1.1 is highlighted grey and would elicit a 'borderline' call, with 'positive' samples above, and 'negative' samples below.



Supplemental Figure 4. Association between time since positive SARS-CoV-2 PCR and IgG avidity index. Plots show results for 2, 4, 5, and 8M urea avidity experiments. For each plot, x-axis displays when sample was collected from an individual after a positive PCR result, and y-axis displays avidity index.



Supplemental Figure 5. Correlation between optical density (OD) ratio to calibrator and avidity index (AI) by different urea wash concentrations. (A) OD ratio versus AI for pre-pandemic samples (2018 NAIIS). (B) OD ratio versus AI for samples from with positive SARS-CoV-2 PCR. For each plot, regression line is displayed as hashed line with regression estimates.



Supplemental Figure 6. Differences in absolute quantity of IgG and avidity indices between pre-pandemic and SARS-CoV-2 PCR positive sample sets.