**Supplementary Information - Humoral immune response to SARS-CoV-2 and endemic coronaviruses in urban and indigenous children in Colombia**

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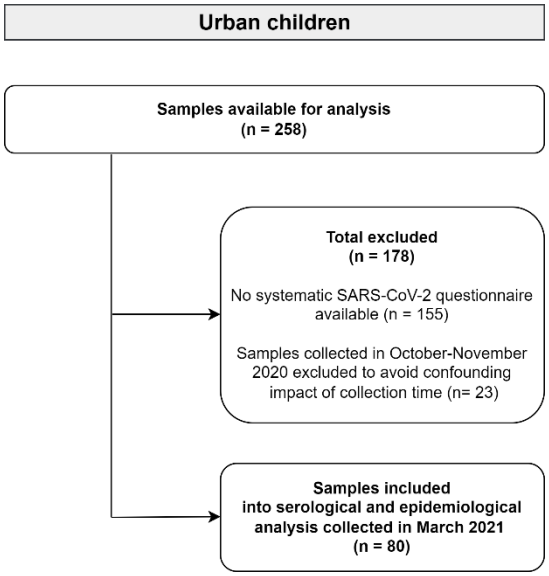
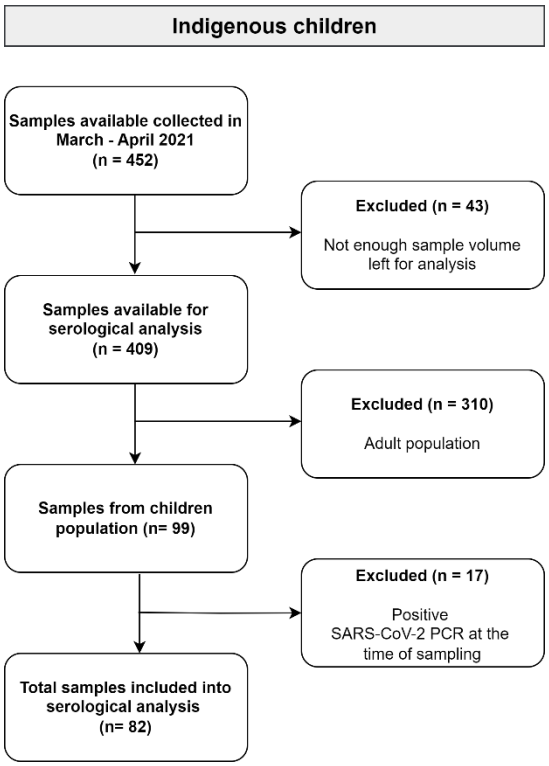
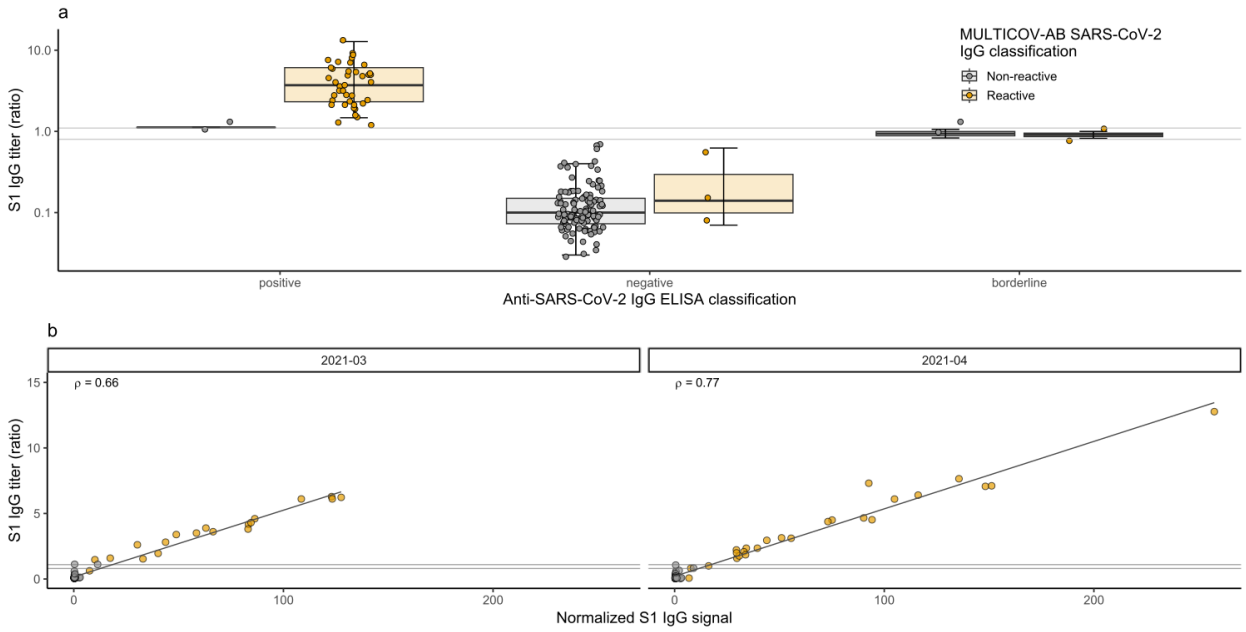



**Supplementary Figure 1.** Sample selection strategy for analysis of SARS-CoV-2 and endemic coronavirus antibody responses in urban children

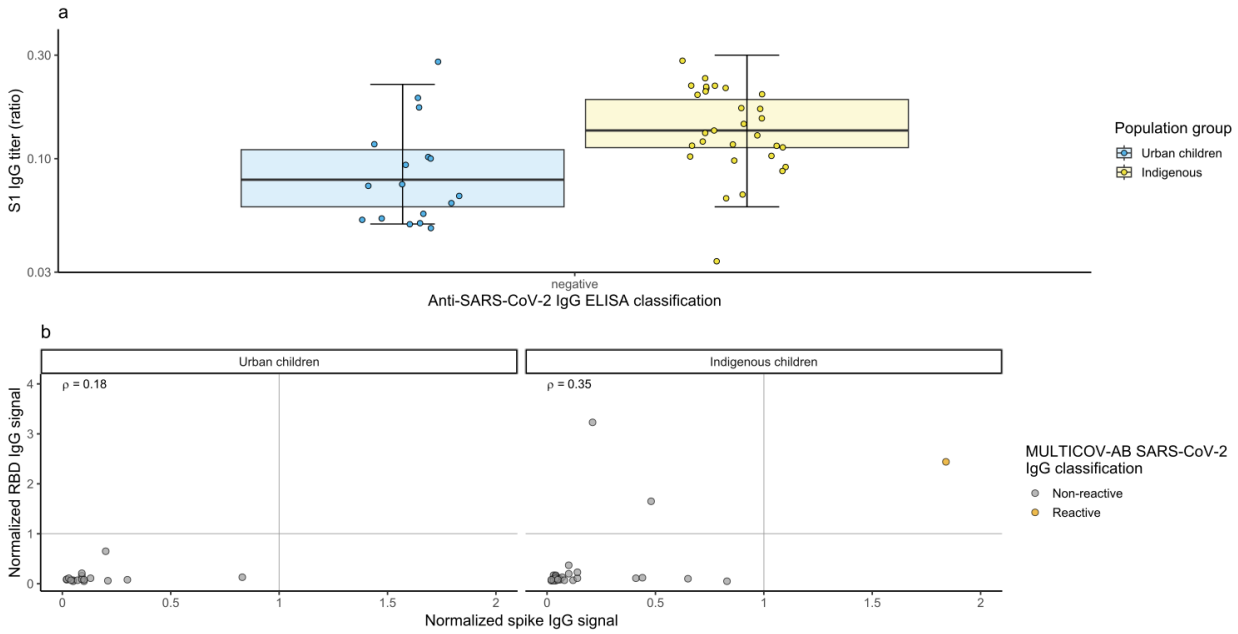



**Supplementary Figure 2.** Sample selection strategy for analysis of SARS-CoV-2 and endemic coronavirus antibody responses in indigenous children

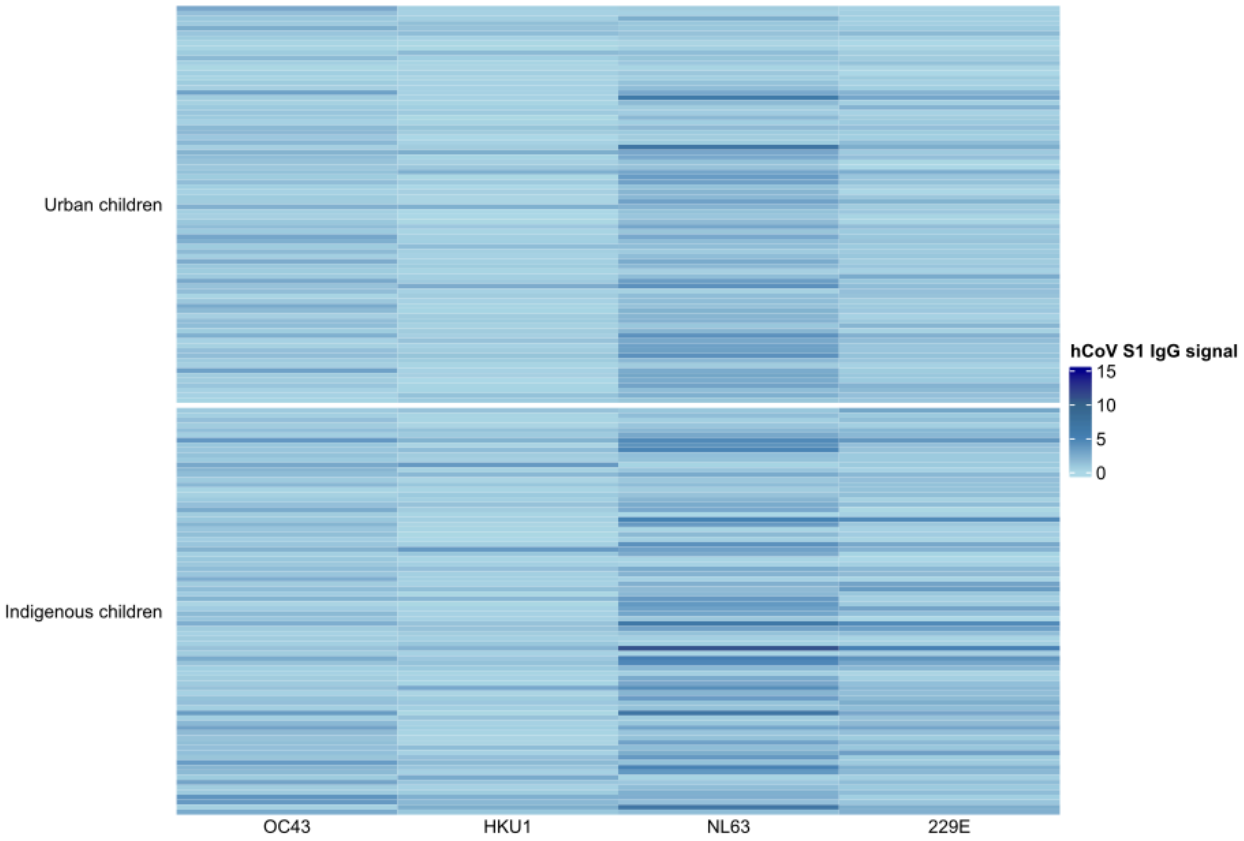



**Supplementary Figure 3.** Comparison between SARS-CoV-2 IgG ELISA and MULTICOV-AB results in the study population (n=162)

**a.** Spike S1-specific serum IgG levels plotted as semi-quantitative S/CO ratio on the y-axis were categorized into the respective qualitative ELISA results (non-reactive, borderline, reactive). Boxes represent the median, 25th and 75th percentiles, whiskers show the largest and smallest non-outlier values. Outliers were determined by 1.5 times IQR. **b.** SARS-CoV-2 S1 IgG titers measured by MULTICOV-AB (x-axis) and EUROIMMUN ELISA (y-axis) were plotted for correlation analysis by Spearman coefficient ρ. Samples in graph b were additionally split based on collection time. Borderline results (ratio: ≥0.8 - <1.1) in the ELISA are located within the grey lines (a, b). Qualitative MULTICOV-AB results per individual sample are indicated by yellow- (reactive) or grey (non-reactive) -colored symbols (a, b).




**Supplementary Figure 4.** Measurement of pre-pandemic indigenous and urban sera samples
Samples from urban children (n=17) and from the Wiwa community (n=30) collected in 2018 and 2019 were measured as specificity control with the EUROIMMUN ELISA (a) and MULTICOV-AB (b). S/CO ratios for the indicated antigens were plotted to illustrate value distribution. Reactivity in the EUROIMMUN ELISA is defined as S/CO ratio ≥1.1 (a) or for MULTICOV-AB as dual S/CO ratio ≥1.0 (b, grey lines) for the indicated antigens. For correlation analysis between antigens, Spearman coefficient ρ was used (b). Qualitative MULTICOV-AB results per individual sample are indicated by yellow- (reactive) or grey (non-reactive) -colored symbols (b). Boxes represent the median, 25th and 75th percentiles. Whiskers show the largest and smallest non-outlier values (a).




**Supplementary Figure 5.** Humoral immune response towards the endemic human coronaviruses in urban and indigenous children

S1 IgG responses towards the endemic coronaviruses OC43, HKU1, NL63 and 229E were measured in children from Bogotá (n=80) and from the indigenous Wiwa (n=82) using MULTICOV-AB. Normalized MFI ratios are plotted as heatmap with color shades from light to dark blue to display increasing S1 IgG signal ratios.

**Supplementary Table 1**. Exclusion factors from the study for children and their companions living in Bogotá

| **Comorbidities or medical conditions** |
|---|
| Companions above the age of 60 |
| Hypertension |
| Obesity |
| Malnutrition |
| Pregnancy |
| Predisposition for bleeding or blood clots |
| Any form of immune disorder |
| Metabolic syndromes (thyroid disease, severe liver or kidney disease, diabetes) |
| Neurologic disorders, cognitive deficits |
| Untreated asthma or other pulmonary diseases |

**Supplementary Table 2.** Characteristics of urban children from Bogotá

| **Characteristics of the participants** | | **80 (100%) All participants: n (%)** | **63 (78.8%) SARS-CoV-2 IgG non-reactive: n (%)*** | **17 (21.2%) SARS-CoV-2 IgG reactive: n (%)*** | **p-value (Fisher's Exact Test)** |
|---|---|---|---|---|---|
| *Socio-economic conditions* | | | | | |
| Age in years: Median (IQR) | | 11 (9-14) | 11 (9-14) | 11 (8-15) | ---§ |
| Sex | Male | 37 (46.3) | 28 (44.4) | 9 (52.9) | p=0.59 |
| | Female | 43 (53.7) | 35 (55.6) | 8 (47.1) | |
| BMI (kg/m$^2$): Median (IQR) | | 19.35 (16.96-21.36) | 18.82 (16.59-21.31) | 19.65 (17.98-22.16) | ---§ |
| School type | Public | 53 (66.3) | 40 (63.5) | 13 (76.5) | p=0.40 |
| | Private | 27 (33.7) | 23 (36.5) | 4 (23.5) | |
| Affiliation to the health system | Subsidized | 8 (10.0) | 8 (12.7) | 0 (0.0) | p=0.19 |
| | Contributory | 72 (90.0) | 55 (87.3) | 17 (100.0) | |
| Socioeconomically strata** | One | 4 (5.0) | 2 (3.2) | 2 (11.8) | p=0.46 |
| | Two | 46 (57.5) | 36 (57.1) | 10 (58.8) | |
| | Three | 29 (36.2) | 24 (38.1) | 5 (29.4) | |
| | Four | 1 (1.3) | 1 (1.6) | 0 (0.0) | |
| Income† | 1-2 minimum wages | 53 (66.2) | 39 (61.9) | 14 (82.3) | **p=0.02‡** |
| | 2-6 minimum wages | 18 (22.5) | 18 (28.6) | 0 (0.0) | |
| | > 6 minimum wages | 3 (3.8) | 2 (3.2) | 1 (5.9) | |
| | Reply denied | 5 (6.2) | 4 (6.3) | 1 (5.9) | |
| | Unknown | 1 (1.3) | 0 (0.0) | 1 (5.9) | |
| Locality | Ciudad Bolívar | 29 (36.2) | 20 (31.7) | 9 (52.9) | p=0.51 |
| | Kennedy | 30 (37.5) | 25 (39.7) | 5 (29.4) | |
| | Bosa | 12 (15.0) | 10 (15.9) | 2 (11.8) | |
| | Tunjuelito | 9 (11.3) | 8 (12.7) | 1 (5.9) | |
| Country | Colombia | 79 (98.7) | 62 (98.4) | 17 (100.0) | p=1.00 |
| | Venezuela | 1 (1.3) | 1 (1.6) | 0 (0.0) | |
| *Variables associated with SARS-CoV-2 infection* | | | | | |
| Participants with PCR-confirmed SARS-CoV-2 infection | Yes | 6 (7.5) | 0 (0.0) | 6 (35.3) | **p=<0.001** |
| | No | 74 (92.5) | 63 (100) | 11 (64.7) | |
| Contact with probable or confirmed COVID-19 case | Yes | 15 (18.7) | 8 (12.7) | 7 (41.2) | **p=0.01‡** |
| | No | 64 (80.0) | 54 (85.7) | 10 (58.8) | |
| | Unknown | 1 (1.3) | 1 (1.6) | 0 (0.0) | |
| Contact with individuals suffering from COVID-19 related symptoms | Yes | 2 (2.5) | 1 (1.6) | 1 (5.9) | p=0.38 |
| | No | 78 (97.5) | 62 (98.4) | 16 (94.1) | |
| Members of the family with confirmed SARS-CoV-2 infection | Yes | 17 (21.3) | 9 (14.3) | 8 (47.1) | **p=0.006** |
| | No | 63 (78.7) | 54 (85.7) | 9 (52.9) | |
| Healthcare worker in the family | Yes | 5 (6.2) | 4 (6.3) | 1 (5.9) | p=1.00 |
| | No | 75 (93.8) | 59 (93.7) | 16 (94.1) | |
| Travel history of minor or household member to location with confirmed COVID-19 cases within 14 days before participation | Yes | 8 (10.0) | 7 (11.1) | 1 (5.9) | p=1.00 |
| | No | 72 (90.0) | 56 (88.9) | 16 (94.1) | |
| Paracetamol use | Yes | 13 (16.3) | 7 (11.1) | 6 (35.3) | **p=0.02** |
| | No | 67 (83.7) | 56 (88.9) | 11 (64.7) | |

* Based on MULTICOV-AB SARS-CoV-2 IgG serostatus

** One corresponds to the lowest and six to the highest strata

† Colombian minimum wage approximate 280 USD

‡ Unknown category and Reply denied category were not included in the calculation

§ Quantitative variables were examined using a logistic regression: Age OR 1.01 per year (95% CI: 0.86-1.19), BMI OR 1.10 per kg/m$^2$ (95% CI: 0.94-1.31)

**Supplementary Table 3.** Self-reported symptoms of urban children from Bogotá

| **Symptoms** | | **80 (100%) All participants n (%)** | **63 (78.8%) SARS-CoV-2 IgG non-reactive n (%) *** | **17 (21.2%) SARS-CoV-2 IgG reactive n (%) *** | **p-value (Fisher's Exact Test)** |
|---|---|---|---|---|---|
| *Respiratory symptoms* | | | | | |
| Fever | No | 76 (95.0) | 59 (93.6) | 17 (100.0) | p=0.57 |
| | Yes | 4 (5.0) | 4 (6.4) | 0 (0.0) | |
| Nasal congestion | No | 64 (80.0) | 52 (82.5) | 12 (70.6) | p=0.31 |
| | Yes | 16 (20.0) | 11 (17.5) | 5 (29.4) | |
| Cough | No | 75 (93.7) | 60 (95.2) | 15 (88.2) | p=0.28 |
| | Yes | 5 (6.3) | 3 (4.8) | 2 (11.8) | |
| Throat pain | No | 75 (93.7) | 60 (95.2) | 15 (88.2) | p=0.28 |
| | Yes | 5 (6.3) | 3 (4.8) | 2 (11.8) | |
| Breathing difficulties | No | 80 (100.0) | 63 (100.0) | 17 (100.0) | --- |
| Loss of smell or taste | No | 79 (98.7) | 63 (100.0) | 16 (94.1) | p=0.21 |
| | Yes | 1 (1.3) | 0 (0.0) | 1 (5.9) | |
| Muscle or bone pain | No | 78 (97.5) | 62 (98.4) | 16 (94.1) | p=0.38 |
| | Yes | 2 (2.5) | 1 (1.6) | 1 (5.9) | |
| *Gastrointestinal symptoms* | | | | | |
| Nausea | No | 75 (93.7) | 61 (96.8) | 14 (82.3) | p=0.06 |
| | Yes | 5 (6.3) | 2 (3.2) | 3 (17.7) | |
| Abdominal pain | No | 74 (92.5) | 60 (95.2) | 14 (82.3) | p=0.11 |
| | Yes | 6 (7.5) | 3 (4.8) | 3 (17.7) | |
| Diarrhea | No | 73 (91.2) | 58 (92.1) | 15 (88.2) | p=0.64 |
| | Yes | 7 (8.8) | 5 (7.9) | 2 (11.8) | |

* Based on MULTICOV-AB SARS-CoV-2 IgG serostatus

**Supplementary Table 4.** MULTICOV-AB antigen panel

| Virus | Antigen | Manufacturer | Cat # |
|---|---|---|---|
| SARS-CoV-2 | spike trimer | NMI | - |
| SARS-CoV-2 | RBD | NMI | - |
| SARS-CoV-2 | S1 domain | NMI | - |
| SARS-CoV-2 | S2 domain | Sino Biological | 40590-V08B |
| SARS-CoV-2 | Nucleocapsid | Aalto Bioreagents | CK 6404-b |
| HCoV-OC43 | S1 domain | NMI | - |
| HCoV-HKU1 | S1 domain | NMI | - |
| HCoV-NL63 | S1 domain | NMI | - |
| HCoV-229E | S1 domain | NMI | - |

**Supplementary Table 5.** RBDCoV-ACE2 antigen panel

| Virus | Antigen | Manufacturer | Cat # |
|---|---|---|---|
| SARS-CoV-2 | RBD Wuhan (B.1) | NMI | - |
| SARS-CoV-2 | RBD Delta (B.1.617.2) | NMI | - |
| SARS-CoV-2 | RBD Omicron (BA.1) | Sino Biological | 40592-V08H121 |
| SARS-CoV-2 | RBD Mu (B.1.621) | NMI | - |
| SARS-CoV-2 | RBD Gamma (P1) | NMI | - |

**Supplementary Table 6.** SARS-CoV-2 seroprevalence adjustment[1] with assay sensitivity and specificity

| Assay | Sensitivity | Specificity |
|---|---|---|
| MULTICOV-AB | 88.3% calculated from 181/205 reconvalescent individuals with a previous PCR-confirmed SARS-CoV-2 infection [2] | 100% calculated with samples from 72/72 uninfected individuals [2] |
| SARS-CoV-2 ELISA (EI 2606-9601G) | 80% calculated from 164/205 reconvalescent SARS-CoV-2-infected [2] | 97.2% calculated from 70/72 uninfected individuals [2] |

**Supplementary Table 7.** R-packages used for statistical analysis and data visualization

| Analysis | R add-on package |
|---|---|
| Fisher's exact test (function fisher.test()) | stats [3] |
| Generalized Linear Models (GLM) with binomial family, logit link, and Maximum-likelihood (ML) estimation | tidyverse [4], MASS [5] |
| Seroprevalence adjustment according to Lang and Reiczigel [1] | asht [6] |
| Fleiss's k statistic with 95% CI | irrCAC [7] |
| Graphical display | gridExtra [8], ComplexHeatmap [9], ggplot2 [10], cowplot [11], ggpubr [12], ggpmisc [13] |

**Supplementary Table 8.** ACE2 binding inhibition responder rates* towards indicated SARS-CoV-2 RBDs in seropositive** children

| % ACE2 binding inhibition responder rates in SARS-CoV-2 seropositive children | | | | | |
|---|---|---|---|---|---|
| | | **RBD B.1** | **RBD ɣ** | **RBD μ** | **RBD o BA.1** |
| **Urban children (17)** | **Responder (n)** | 4 | 0 | 0 | 0 |
| | **Responder (%)** | **23.5** | **0.0** | **0.0** | **0.0** |
| **Indige-nous children (28)** | **Responder (n)** | 7 | 1 | 1 | 0 |
| | **Responder (%)** | **25.0** | **3.6** | **3.6** | **0.0** |

* Responders are defined with an ACE2 binding inhibition of >20%

** Based on MULTICOV-AB SARS-CoV-2 IgG serostatus

**Supplementary References**


1. Lang, Z. & Reiczigel, J. Confidence limits for prevalence of disease adjusted for estimated sensitivity and specificity. *Preventive Veterinary Medicine* **113**, 13-22 (2014).
2. Becker, M.*, et al.* Exploring beyond clinical routine SARS-CoV-2 serology using MultiCoV-Ab to evaluate endemic coronavirus cross-reactivity. *Nature Communications* **12**, 1152-1152 (2021).
3. Team, R.C. R: A Language and Environment for Statistical Computing. (R Foundation for Statistical Computing, Vienna, Austria, 2021).
4. Wickham, H.*, et al.* Welcome to the Tidyverse. *Journal of Open Source Software* **4**, 1686-1686 (2019).
5. Ripley, W.N.V. & B, D. *Modern Applied Statistics with S*, (Springer, New York, 2002).
6. Fay, M.P. asht: Applied Statistical Hypothesis Tests. (2022).
7. Gwet, K.L. & PhD. Computing Chance-Corrected Agreement Coefficients (CAC). (2019).
8. Auguie, B. gridExtra: Miscellaneous Functions for "Grid" Graphics. (2017).
9. Gu, Z., Eils, R. & Schlesner, M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* **32**, 2847-2849 (2016).
10. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*, (Springer-Verlag New York, New York, 2016).
11. Wilke, C.O. cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'. (2020).
12. Kassambara, A. ggpubr: 'ggplot2' Based Publication Ready Plots. (2020).
13. Aphalo, P.J. ggpmisc: Miscellaneous Extensions to 'ggplot2'. (2022).