Pre-pandemic humoral immunity to SARS-CoV-2 in Africa: systematic review and meta-analysis

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SUMMARY (299 words)

Background: Low numbers of recorded COVID-19 deaths in Africa may be due to undercounting and/or protection due to demographic and/or other factors, including pre-existing immunity. Several studies have assessed pre-pandemic samples for anti-SARS-CoV-2 antibodies with heterogeneous results.

Methods: We performed a systematic review and meta-analysis of studies evaluating pre-pandemic African samples for anti-SARS-CoV-2 antibody activity using pre-set assay-specific thresholds for seropositivity. Data where assay thresholds were calibrated on African populations were excluded. Searches used PubMed (September 13, 2022), reference lists of retrieved papers and citing articles (Google Scholar). Data were extracted independently by two authors on study and assay characteristics, and number of positive and tested samples. Datasets were classified according to malaria, dengue, and HIV burden. Proportions of seropositivity were combined with random effects meta-analysis.

Results: 22 articles with 117 datasets were eligible, including 2,971 positives among 21,988 measurements (13.5%) with large between-dataset heterogeneity. Positivity was higher for anti-S1 (25%) and lower for anti-RBD antibodies (8%). Positivity was non-significantly higher for IgM than for IgG antibodies. Positivity was seen prominently in countries where malaria transmission occurs throughout and in datasets enriched in malaria cases (17%, 95% CI, 15-19%) versus 1%, 95% CI 0-2% in other datasets). There were modest differences according to dengue burden (15% versus 11%). Substantial SARS-CoV-2 reactivity was seen in high malaria burden with or without high dengue burden (seropositivity 19% and 12%, respectively), and not without high malaria burden (seropositivity 2% and 0% with and without high dengue burden, respectively). There were modestly lower proportions of positivity in datasets with >10% of

HIV-infected participants (8% versus 15% in others), but no association according to HIV serostatus in individual samples (summary odds ratio 0.97, 95% CI, 0.55-1.70).

Interpretation: Pre-pandemic samples from Africa show high levels of anti-SARS-CoV-2 seropositivity that tracks especially with malaria.

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RESEARCH IN CONTEXT

Evidence before this study

Several studies have evaluated the presence of antibodies cross-reactive to SARS-CoV-2 in samples collected before the COVID-19 pandemic from African locations. Positivity rates have varied substantially and different hypotheses have been raised about the correlates, causes, and clinical implications of this pre-existing humoral immunity. A search in PubMed and Google Scholar did not identify, however, any systematic review and meta-analysis of existing studies.

Added value of this study

A formal systematic review and meta-analysis identified 22 studies with 117 datasets from pre-pandemic samples from Africa and found that on average 1 of 7 samples had anti-SARS-CoV-2 antibody activity, with large between-dataset heterogeneity. The strongest factor correlating with high positivity rates was malaria, while associations with dengue and HIV were not strong and were probably confounded.

Implications of all the available evidence

Further studies should examine whether pre-existing immunity is related to the lower recorded COVID-19 deaths in settings with high malaria burden. The broader spectrum of immune response in pre-pandemic samples, including both humoral and cellular immunity, should also be carefully dissected.

INTRODUCTION

The number of recorded COVID-19 deaths in Africa during the pandemic has been very small compared with other continents. A wide range of explanations has been proposed,[1] ranging from favorable population features (very young, low obesity rates) to speculations that deaths may have been undercounted in Africa due to limited testing.[2] One of the most tantalizing hypotheses has been that pre-existing immunity to SARS-CoV-2 may account for less severe clinical outcomes and fewer fatalities. Impetus for this hypothesis has been provided by several studies documenting humoral immunity to SARS-CoV-2 in African samples that predate the pandemic years.[3-12] This pattern has typically not been seen in populations from highly developed countries and its provenance remains elusive. This detected immunity does not seem to represent cross-reactivity of immune responses to known endemic coronaviruses that are extremely widely, practically ubiquitously, distributed across all continents. There have been speculations whether detected anti-SARS-CoV-2 antibodies represent prior exposure to some other, yet unknown, coronavirus bearing similarity to SARS-CoV-2, or may be related to exposure to other infectious pathogens widespread in Africa, in particular *Plasmodium*, dengue and HIV.

African studies on this matter have used very diverse sources of pre-pandemic samples in various countries. They have also used different antibody assays for various antibody types and SARS-CoV-2 antigenic targets. There are conflicting hints on whether cross-reactivity may pertain mostly to specific antibody types and antigenic targets. Some studies have also explored different correlations between the presence of anti-SARS-CoV-2 antibodies and markers of various other infectious diseases. While each of them offers a limited picture, a systematic examination may offer some more concrete insights. Important questions to answer are: how

frequently are antibodies to SARS-CoV-2 detected in pre-pandemic African samples, how much heterogeneity exists on their prevalence, and whether heterogeneity may be explained by the assay and type of antibodies assessed, each country's endemicity for *Plasmodium* and dengue, HIV infection rates, and any other associations with infectious pathogens. This systematic review and meta-analysis aimed to answer these questions.

METHODS

Protocol

The protocol was preregistered in OSF (https://osf.io/f5g76/).

Eligible studies

Eligible studies were those that evaluated samples collected in African countries before December 2019, i.e. in the pre-pandemic period, for humoral responses to SARS-CoV-2 in blood (plasma or serum). Any type of antibody and any type of assay thereof was eligible. Studies were eligible if they used pre-set assay-specific thresholds of antibody titers for claiming positive results. Studies that measured antibodies in other body fluids (e.g., milk) were excluded. Studies that calibrated the assay threshold so as to make it appropriate for use in African populations by setting the specificity at a desired level were excluded; however, if these studies presented also specificity results according to an original pre-set threshold (based on previous work on other, non-African populations), the estimates of specificity based on the pre-set threshold were eligible. Similarly, studies that used assays that were previously calibrated so as to have a desirable level of specificity in African samples were excluded. Furthermore, studies that considered only pre-pandemic samples that were already screened to be negative for anti-SARS-CoV-2 antibodies based on some other SARS-CoV-2 antibody assay were excluded. For studies that included both African and other continent samples from the pre-pandemic era, only the

former were eligible, and data were thus considered only if the African set could be separated. We did not consider neutralizing antibody assays, as it has been clearly shown that detected antibodies in pre-pandemic African samples typically do not have neutralizing capacity.[13,14]

Search strategies

PubMed was searched (last update September 13, 2022) with the following search string: (Nigeria OR Ethiopia OR Egypt OR Congo OR Tanzania OR South Africa OR Kenya OR Uganda OR Sudan OR Algeria OR Morocco OR Angola OR Mozambique OR Ghana OR Madagascar OR Cameroon OR Cote d'Ivoire OR Niger OR Burkina Faso OR Mali OR Malawi OR Zambia OR Senegal OR Chad OR Somalia OR Zimbabwe OR Guinea OR Rwanda OR Benin OR Burundi OR Tunisia OR South Sudan OR Togo OR Sierra Leone OR Libya OR Liberia OR Central African Republic OR Mauritania OR Eritrea OR Namibia OR Gambia OR Botswana OR Gabon OR Lesotho OR Guinea-Bissau OR Africa OR African) AND (pre-pandemic OR prepandemic OR cross-reactiv* OR seroprevalence OR negative samples OR negative controls OR (specificity AND test) OR (specificity AND antibod*)) AND (COVID-19 OR SARS-CoV-2). We also searched the reference lists of the retrieved eligible papers and searched in Google Scholar the articles that cite the retrieved eligible papers in order to identify and additional relevant eligible papers.

Data extraction

From each eligible paper, we extracted the following information: first author, publication venue, African county(ies) from which pre-pandemic samples were obtained, sample size (per country and per cohort, if many countries/cohorts were assessed), provenance of the samples and any information about the sampling process, time periods when they were collected, age information; type of SARS-COV-2 assays and of antibodies measured, including

manufacturer or laboratory of provenance, type of antibody (IgG, IgM, IgA, combinations, all antibodies) and antigenic targets (spike S, S1 subunit, S2 subunit, nucleocapsid N, receptor binding domain RBD, receptor binding motif RBM, other); number and percentage of positive samples for each antibody/assay assessed among total assessed; any borderline readings; any additional information on measurements of indicators of other infectious diseases (*Plasmodium* parasitemia, *Plasmodium* antigens, HIV positivity status, dengue, other) with the potential to generate 2x2 tables for SARS-CoV-2 antibodies against these indicators; and any additional information on relationships between SARS-CoV-2 antibodies and other factors assessed by the authors.

Data extraction was performed in duplicate by two independent assessors who then compared notes and solved discrepancies with discussion.

Risk of bias assessment

The eligible articles were assessed using the Joanna Briggs risk of bias tool for prevalence studies that includes 9 assessment items.[15] The assessment was done by one assessor.

Meta-analysis

The available data on percentage of positive samples with different assays and antigenic targets were combined with meta-analysis. Data were combined separately for each antigenic target using a random effects model.[16]

Heterogeneity was assessed with the chi-square-based Q test and with the I^2 statistic.[17] Borderline readings were considered negative (as also done by the original individual study authors).

Subgroup analyses were performed according to type of antibody (IgG, IgM, IgA, combinations, all antibodies). Moreover, when different types of antibody (e.g. IgG and IgM)

had been assessed with the same assay platform in the same samples, the odds ratio of positivity was determined; if information were given on how many samples were positive with both antibody types (P1P2), how many samples are negative with both antibody types (N1N2), and how many samples are positive with only one of the two antibody types (P1N2, N1P2), the matched McNemar odds ratio was calculated as the ratio P1N2/N1P2.

We also performed subgroup analyses according to country of origin of the samples, classifying countries according to endemicity/burden for malaria, dengue, and rates of HIV infection. For malaria, we used the CDC map of malaria distribution (https://www.cdc.gov/malaria/about/distribution.html)[18] separating countries where malaria transmission occurs throughout versus those where no transmission occurs or transmission occurs only in some places. For dengue, we used the Global Consensus 2013 map (https://www.healthmap.org/dengue/en/)[19] separating countries where dengue is present or likely from countries where dengue in uncertain, unlikely or absent. For HIV, we considered the eight countries with highest HIV positivity rates (>10% according to https://en.wikipedia.org/wiki/List_of_countries_by_HIV/AIDS_adult_prevalence_rate,[20] i.e. Botswana, Lesotho, Mozambique, Namibia, South Africa, Eswatini, Zambia, and Zimbabwe) versus others. Studies where sampling explicitly aimed to recruit participants based on or heavily enriched in *Plasmodium*, dengue, or HIV infections were considered in the groups with high burden, even if they come from countries without high burden.

Finally, whenever data were available to generate 2x2 tables for the presence of anti-SARS-CoV-2 antibodies and other infectious disease markers or other factors potentially associated with such antibodies, random effects meta-analyses were also performed for the odds ratios of each probed association across the eligible studies that presented sufficient data.

Meta-analyses were conducted in STATASE 15. P-values were considered statistically significant for P<0.005 and suggestive for values between 0.005 and 0.05.[21]

RESULTS

Eligible studies

After screening 38 studies (Figure 1) and excluding 16 (Supplementary references), 22 studies were eligible (18 retrieved from the main search and another 4 retrieved from cited/citation searches).[3-14, 22-31] 19 studies were published in peer-reviewed journals and 3 were preprints. Other studies screened in-depth and excluded are shown in Supplementary References along with the reason for exclusion. Supplementary Table 1 shows the results of the risk of bias assessment.

As shown in Table 1, with one exception participants came from Sub-Saharan Africa. Dataset sample sizes ranged from 19 to 1,077 (sum=21,988). There was also wide variation in the settings of sample collection and eligibility criteria. Five studies included independent cohorts from more than one country and one study included 4 different cohorts from the same country. The total number of cohorts was 38. Most samples were selected between 2016 and 2019, but 8 cohorts had earlier samples (earliest, 1999). Twelve cohorts included only adults, 17 had collected samples from both children and adults, and 2 included only children (7 had unclear age distribution).

As shown in Table 2, there was a large variety of assays used, but most studies used assays of IgG. Most (13/22) studies assessed antibodies against both spike and nucleocapsid antigenic targets. 5 used only antibodies against spike targets and 4 used only antibodies against nucleocapsid targets. In-depth assessments for associations with indicators of infectious pathogens were sparse.

Positivity for SARS-CoV-2 antibodies

Supplementary Table 2 shows the data on the presence of positive results for the assessed SARS-CoV-2 antibodies and variables considered in the subgroup analyses. Overall, 117 datasets among pre-pandemic samples were available for analysis with crude total of 2,971 positives among 21,988 measurements (13.5%). When all 117 datasets were considered in meta-analysis, there was large between-dataset heterogeneity ($p<0.001$, $I^2=97.2\%$) with summary random effects positivity 14% (95% CI, 12-15%).

Meta-analysis of positivity per antigenic target and antibody type

As shown in Table 3, summary positivity was very similar (16%) for anti-N and anti-S antibodies. However, when more specific epitopes within spike were considered, summary positivity was higher for anti-S1 (25%, 95% CI, 19-30%) and lower for anti-RBD antibodies (8%, 95% CI, 6-10%). Lowest positivity was seen for antibodies using both N and S (or subtype) targets (6%, 95% CI, 3-9%). Positivity was non-significantly higher for IgM (summary 16%) than for IgG (summary 13%) antibodies. There was very large between-dataset heterogeneity in all summary estimates ($p<0.001$), so they should be seen with great caution.

Paired comparisons of antibody types

In 15 datasets, both IgG and IgM had been measured with the same type of assays and antigenic target on the same samples. For 13/15, data on paired measurements in each sample were available (Supplementary Table 3).: the summary paired odds ratio was 1.02 (95% CI, 0.86-1.22, $I^2=75\%$). Another 2 small datasets had no sufficient data to calculate paired odds ratios.

Subgroup analyses for malaria, dengue, and HIV

As shown in Table 3, anti-SARS-CoV-2 positivity was very different according to malaria status. It was seen prominently in countries where malaria transmission occurs throughout and in datasets enriched in malaria cases (summary estimate 17%, 95% CI, 15-19%), but was almost absent in other datasets (summary estimate 1%, 95% CI, 0-2%). There was still very large between-study heterogeneity in the former group, and less heterogeneity in the latter. Among the 22 datasets in the latter group, positivity was always 0% or very low (=<8%) except for one dataset from Tanzania which is a country where malaria transmission does occur throughout in altitudes below 1800 meters.

A higher proportions of anti-SARS-CoV-2 positivity was seen in high dengue burden datasets than others (summary estimates 15% versus 11%), but large between-dataset heterogeneity existed within each group ($p<0.001$). All the low-dengue datasets with non-negligible positivity came from studies in populations with high levels of malaria. In datasets from three countries with present/likely dengue but malaria transmission not occurring throughout (Tanzania, Magadascar, Ethiopia) the summary positivity rate was only 2% (95% CI, 1-3%). Excluding Tanzania (where, as above, malaria transmission occurs throughout in many parts of the country), estimates for Ethiopia and Magadascar combined were 0% (95% CI, 0-1%) with no between-dataset heterogeneity ($p=0.98$, $I^2=0\%$). Substantial SARS-CoV-2 reactivity was seen practically only in subgroups with high malaria burden with or without high dengue burden (seropositivity 19% and 12%, respectively), not in subgroups without high malaria burden (seropositivity 2% and 0% with and without high dengue burden, respectively) (Figure 2).

An inverse association was seen with HIV infection, with lower proportions of anti-SARS-CoV-2 antibody positivity in countries with high HIV transmission or datasets with >10%

of the sample being HIV-positive than in datasets with lower HIV rates (summary positivity 8% versus 15%). There was large between-dataset heterogeneity ($p<0.001$) within each group.

Other assessed associations with infectious disease indicators

One study[11] provided data on positivity with 4 different anti-SARS-CoV-2 antibodies in subgroups defined by the presence of *Plasmodium* parasitemia. Combining the 4 evaluations (Supplementary Table 4), the summary odds ratio was 1.84 (95% CI, 0.90-3.78, I^2=29.2%). Two studies[9,12] included data from 3 cohorts where presence of anti-SARS-CoV-2 antibodies was given per HIV serostatus (Supplementary Table 5); the summary odds ratio was 0.97 (0.55-1.70, I^2=0%). Data were limited or not presented in sufficient detail for formal meta-analysis for other infectious disease indicators.

DISCUSSION

The present meta-analysis includes data from 22 studies with 117 datasets and more than 20,000 measurements of anti-SARS-CoV-2 antibodies in pre-pandemic samples from Africa. On average, 1 of 7 samples tested positive for anti-SARS-CoV-2 antibodies, but there was extensive heterogeneity across studies and datasets, with several studies finding 0% positivity and some others exceeding 80%. Cross-reacting immunity was slightly more common with IgM rather than IgG measurements on average, but prominence of IgM versus IgG signals varied greatly across datasets. While spike and nucleocapsid antibodies overall did not have substantial differences in positivity on average, subtypes with spike had different profiles with higher positivity for antibodies to the S1 domain rather than for antibodies to the RBD domain. Stark differences were seen according to malaria burden. Anti-SARS-CoV-2 positivity was seen almost entirely in samples from areas with malaria transmission throughout and/or enriched in malaria cases. A more modest association was seen for dengue. However, malaria and dengue endemicity largely

overlap, and samples coming from high dengue but low malaria burden settings had negligible positivity. Finally, HIV was associated with modestly lower frequency of anti-SARS-CoV-2 antibodies. This may have reflected mostly, if not entirely, the inverse geographical localization of HIV and malaria burden in Africa.

The composite picture is consistent with the possibility that the observed pre-pandemic humoral immunity to SARS-CoV-2 in Africa may reflect mostly cross-reactive response to malaria. Lapidus et al. suggest that this immune response is more common and more intense in acute and recent malaria.[14] The large between-dataset heterogeneity in positivity among studies with high malaria burden may reflect the large variability in the magnitude of that burden, with some datasets including exclusively acute malaria, others being heavily enriched in malaria cases, and others simply coming from areas with substantial malaria burden. Cross-reactivity with dengue is also possible, but most areas in Africa that have high dengue burden have also high malaria burden. Analysis of datasets from settings with high dengue but low malaria shows negligible anti-SARS-CoV-2 positivity. An elusive, previously unidentified coronavirus that circulated in parts of Africa in the past cannot be excluded, but it is unnecessary to invoke to explain the observed cross-reactive immunity. If it existed, such a pathogen may have largely shared the geography of malaria.

Anti-SARS-CoV-2 humoral immunity in pre-pandemic samples has generally not been observed in European and USA studies. Data from countries outside Africa also suggest that malaria rather than dengue has a strong association with the presence of antibodies that are cross-reactive against SARS-CoV-2. Manning et al.[32] found 14% cross-reactive positivity among 528 samples of patients with malaria from Cambodia. Data for dengue are mixed. One study in Taiwan[33] found higher optical density anti-S1 RBD activity in archival dengue samples than in

controls, but the optical density values were still low. Another study[34] found some IgM and IgA rather IgG false-positivity for SARS-CoV-2 in febrile illness from dengue in Thailand, but the false-positivity tended to be even more frequent for febrile illness from non-dengue cases (including apparently malaria). In a study with samples from Puerto Rico and USA,[35] dengue did not induce cross-reactive antibodies to SARS-CoV-2 and the same was true in dengue samples from Indonesia,[36] Colombia[37] and travel clinics.[38] Conversely, 5 of 17 archival dengue samples from India[39] had cross-reactive antibodies to SARS-CoV-2 and another study[40] found 22% cross-reactivity in samples from an Israel center (unspecified country of provenance); however, it is unknown whether any positive samples could be from patients who also had a history of malaria. SARS-CoV-2 infection has been described to frequently produce cross-reactive antibody activity to dengue,[36,37,40] but not seen in all studies.[35] Anti-SARS-CoV-2 antibodies may also have a protective role for dengue[33] and, interestingly, reported dengue cases and deaths have declined in 2020-2022 after a peak in 2019.[41] In-silico analysis shows possible similarities between SARS-CoV-2 epitopes in the HR2 domain of the spike protein and the dengue envelope protein,[40] but the evidence is again stronger for malaria, where cross-reactive antibodies specifically recognized the sialic acid moiety on N-linked glycans of the Spike protein.[14]

The clinical and public health importance of pre-existing humoral immunity remains a tantalizing question. Typically, the detected antibodies test negative in neutralization assays.[13,14] However, they may be a marker of a much broader immune response that includes both humoral and cellular features. Pre-existing T-cell immunity and its potential role in ameliorating clinical course in SARS-CoV-2 infection is another hotly debated issue.[42] It would be useful to assess pre-pandemic samples with anti-SARS-CoV-2 antibodies for a broad spectrum of immune

functionalities. Non-humoral immunity elements may be even more frequent than the detected humoral immunity, since humoral immunity tends to wane relatively rapidly with time.[43]

The geographical pattern of the documented impact of COVID-19 in Africa is intriguingly well aligned with the geographical pattern of detected pre-pandemic immunity. Recorded COVID-19 deaths have been far higher in South Africa (high HIV, relatively low malaria burden) and in northern African countries (low malaria burden) than in other countries in sub-Saharan Africa (high malaria burden). Differences in the extent of under-ascertainment of COVID-19 deaths, demographic and lifestyle differences (older populations in northern Africa, high levels of obesity in South Africa), and many other factors may explain in part or in whole these differences. However, a contribution of pre-existing immunity remains also an additional possibility. Pre-existing immunity has also been raised as a possible important contributing factor to low fatalities in East Asia,[44] and many areas in East Asia also have substantial malaria burden. Conversely, recent dengue outbreaks did not seem to protect from COVID-19 fatalities; the highest number of dengue cases and deaths in 2019 was seen in Brazil,[41] a country that suffered high COVID-19 fatalities.

Some limitations of our work should be discussed. First, the examined studies mostly used convenient samples available from pre-pandemic efforts not tailored specifically to answer questions posed by the pandemic. For many samples, information about their provenance and features was limited. Second, several of our analyses have ecological designs, e.g. when countries were assigned to high or low burden groups for specific pathogens. The observed associations may not necessarily hold true also at the level of analyses profiling prior infection in single individual samples. Nevertheless, the more limited individual level data available also agree with the main findings regarding malaria and HIV. Third, some analyses include datasets

which represent the same samples tested with different assays, therefore they are not entirely independent. However, the major differences observed (e.g. with malaria) remain strong even if only one dataset is selected per study/cohort (not shown). Fourth, it is uncertain whether publication biases may exist for the research questions addressed, e.g. if more studies that found no seropositivity in pre-pandemic African samples may have remained unpublished compared with studies that found high positivity. Fifth, the assays used were very diverse and the technical competence of their performance by different teams cannot be validated independently. This may explain also part of the observed large between-dataset heterogeneity. However, errors would tend to weaken observed associations, if anything, through non-differential mis-classification.

Acknowledging these caveats, our meta-analysis provides strong evidence for pre-pandemic humoral immunity to SARS-CoV-2 in Africa, closing tracking with malaria. Further studies of broader immunological profiles involved and of the public health implications are necessary.

Contributors

Both authors conceived the original idea, wrote the protocol, extracted data, run and interpreted analyses, wrote and revised the paper and approved the final version.

Declaration of interests

No conflicts of interest.

Data sharing statement

All data are in the manuscript and its supplements

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Figure 1: Flow chart for searches and eligible studies

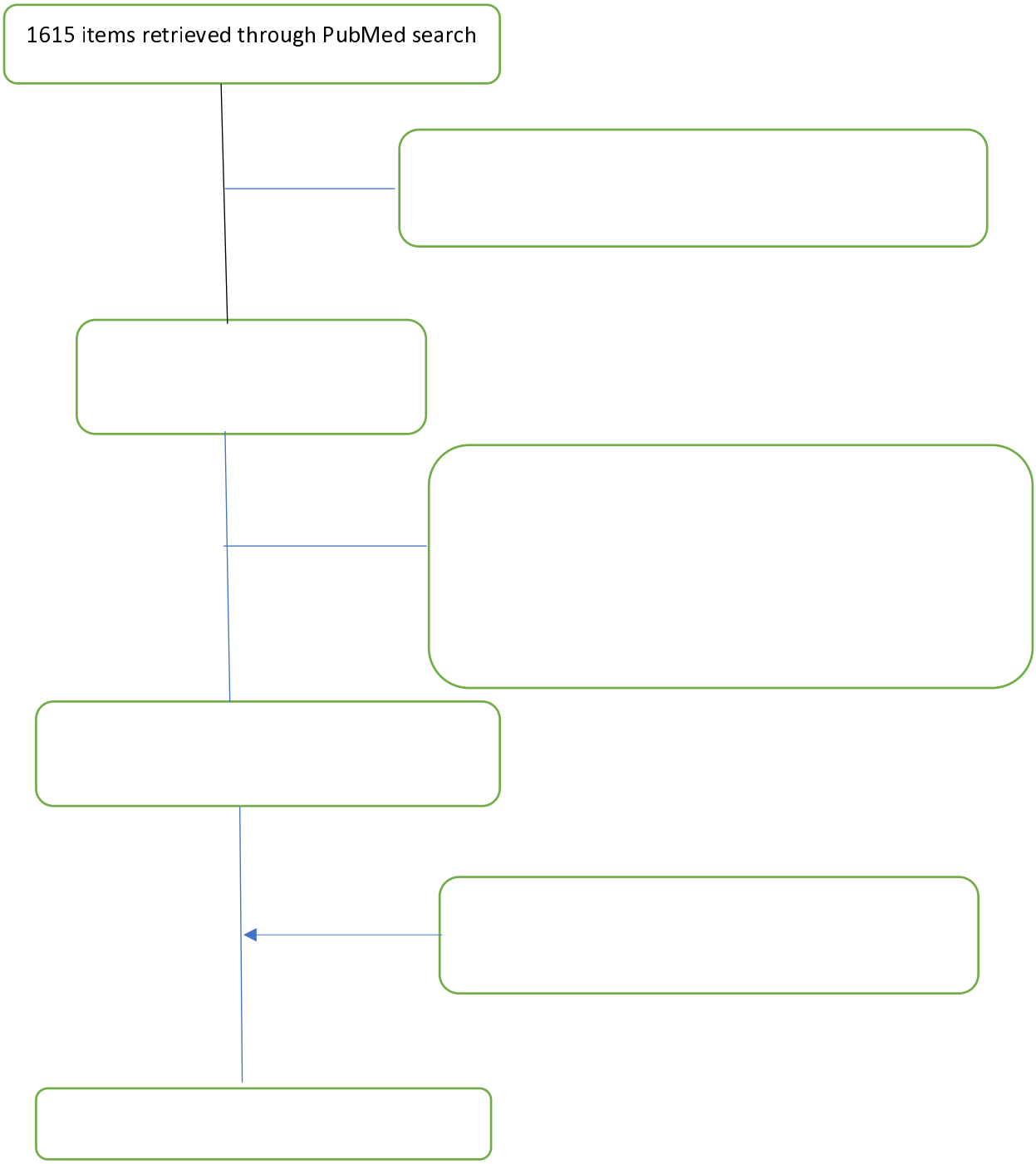

Figure 2: Meta-analysis of positivity rates for anti-SARS-COV-2 antibodies in pre-pandemic samples for studies from countries or settings with high malaria burden only, high malaria and high dengue burden, low malaria and high dengue burden, and neither malaria nor dengue high burden.

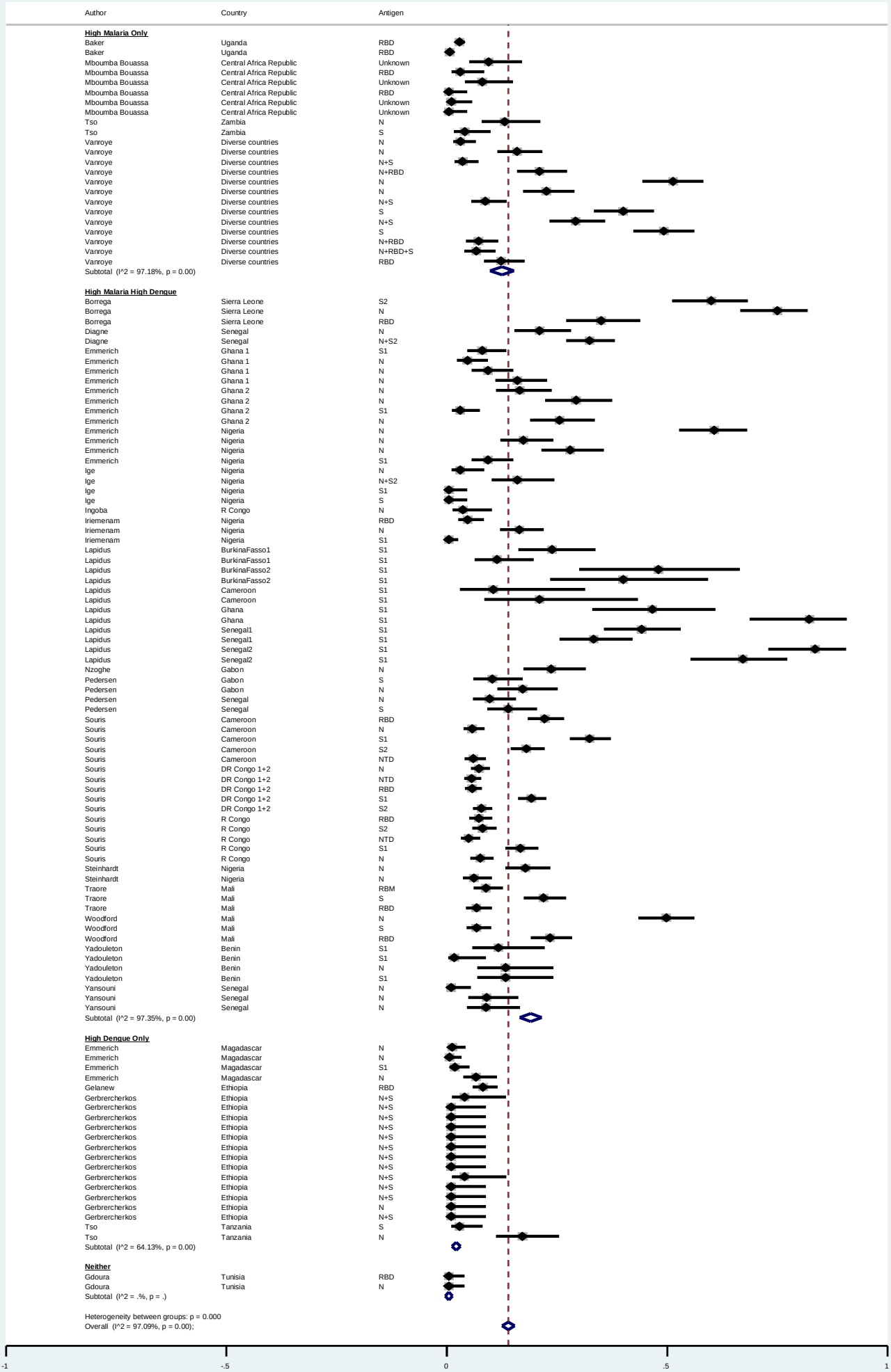

Table 1. Characteristics of eligible studies

Author	Country	Sample size	Provenance of the samples and sampling process	Time period of sample collection	Age in years (median)
Pedersen	Gabon	146	Unclear	Oct 2019	18-50
	Senegal	150	Unclear	Jan 2017-May 2018	65% <18
Borrega	Sierra Leone	120	Lassa and Ebola survivors and their contacts	Sept 2016-April 2019	8-60 (31)
Tso	Tanzania	105	Blood donors, 6.7% HIV-positive	Mar 2019-May 2019	≥18
	Zambia	99	Enriched in HIV-positive (43.4%)	2017- early 2019	≥18
Emmerich	Magadascar	167	Pregnant women	2010	20-30 (23)
	Ghana	150	Children	2014-2015	3-7 (6)
	Ghana	133	Teens and adults	1999	16-45 (22)
	Nigeria	150	Adults	2018	30-58 (41)
Yadouleton	Benin	60	Acute febrile illness, tested for hemorrhagic fever surveillance	Oct 2019 – Nov 2019	12-65 (28)
Nzoghe	Gabon	135	Healthy healthcare worker volunteers	2014	14-80 (38)
Woodford	Mali	312	Urban healthy adults	Jan 2017	≥18
			Rural healthy adults	May 2019	≥18

			Rural women of childbearing age	May 2019	≥18
			Rural all ages	May 2018	All ages
Baker	Uganda	1077	Rakai Community Cohort Study (543 febrile within one month, 534 non-febrile matched for age and gender)	2011-2013	18-54 (30)
Souris	DR Congo	190	Healthy subjects (hospital staff and volunteers) and young sickle-cell disease patients	2019	Any age
	DR Congo	383	Biobank from Plasmodium study in Kinshasa	2014 – 2015	Any age
	Cameroon	383	Continual health monitoring project for HIV patients	Jun 2018 – Jun 2019	Any age
	R Congo	384	Research samples from two districts	2016, 2019	Any age
Ige	Nigeria	100	50 HBV-positive (S-antigen) and 50 HIV-positive	Before Oct 2019	(35)
Traore	Mali	283	Malaria survey in Dangassa village	2019	Unclear
Ingoba	R Congo	82	Bomassa village	Jun- Jul 2019	Unclear
Diagne	Senegal	272	Biobank	Before Sept 2019	Unclear

Yansouni	Senegal	100	Clinical suspects for malaria	Before Jul 2019	Unclear
Iriemenam	Nigeria	213	Nigeria HIV/AIDS Indicator and Impact Survey	2018	0-60 (15)
Steinhardt	Nigeria	213	Nigeria HIV/AIDS Indicator and Impact Survey	2018	0-60 (15)
Gelanew	Ethiopia	365	Pre-pandemic sera	2012-2018	Unclear
Gebrecherkos	Ethiopia	50	Pre-pandemic samples from patients with other infections	2017	Unclear
Mboumba Bouassa	Central African Republic	100	National center of sexually transmitted diseases in Bnngui, 54 HIV-seropositive, 5 HCV-positive, 35 HBsAg-positive	2000-2011	(31 mean)
Gdoura	Tunisia	116	Pre-pandemic samples	2017	Unclear
Vanroye	Diverse African	195*	Travelers from Africa with malaria or schistosomiasis	2010-2018	8-61 (~40)
Lapidus	Cameroon	19	Malaria patients	Jul-Nov 2018	2-64 (26 mean)
	Senegal 1	120	Malaria patients	Jul 2019	1-74 (22 mean)
	Senegal 2	67	Malaria patients	2015-2017	5-16 (11 mean)
	Burkina Fasso 1	88	Malaria patients	Jul-Aug 2017	0-4 (3 mean)
	Burkina Fasso 2	25	Malaria patients	Oct 2016-Feb 2017	21-43 (33)

	Ghana	45	Malaria patients	Jul 2007- June 2010	3-70 (15)

*Includes 9 samples from Asia and 19 of unknown country origin; besides the n=195, the study has 25 patients with dengue, but few are from Africa, so only the malaria and schistosomiasis cases are considered here

Table 2. Immunological assays performed in the eligible studies and other pathogen indicators assessed

Author	Antibody assays used	Antigenic targets	Other pathogen indicators assessed
Pedersen	ELISA	S, N	
Borrega	reSARS, cutoffs defined by USA samples	N, RBD, S2	
Tso	Immunofluorescence assay, with USA control samples	N, S	HIV
Emmerich	Euroimmun, EDI, Mikrogen recomWell	S1 IgG (Euroimmun), N IgG (Euroimmun), N IgG (EDI), N IgG (Mikrogen)	*Plasmodium* parasitemia
Yadouleton	Euroimmun, INBIOS	S IgG (Euroimmun), S1 IgA (Euroimmun), S1 IgG (INBIOS), N IgG (Euroimmun)	
Nzoghe	Elecsys Roche	N all subclasses	Multiple infectious indicators assessed but presented only for SARS-C0V-2 antibody-positive samples
Woodford	Previously developed at NIH with USA samples	S, N, RBD	*Plasmodium* antigens
Baker	CoronaCHEK	Spike RBD IgG or IgM	HIV
Souris	INNOBIOCHIPS ELISA,	S1, S2, S1-RBD, S1-	

	calibrated on other human coronaviruses	NTD, N	
Ige	Euroimmun, Mologic, Abbott Architect	S1 IgG (Euroimmun), N IgG (Euroimmun), S2 or N IgG (Mologic), N IgG (Abbott)	
Traore	ELISA	S IgG, RBD IgG, RBM IgG	*Plasmodium* parasitemia, *Plasmodium* smear
Ingoba	Virotech, according to kit manufacturer	N IgG (also IgM but no data given)	
Diagne	Omega, ID-screen	N or S2 IgG (Omega), N IgG (ID-screen)	*Plasmodium* and other pathogen antibodies
Yansouni	Abbott Architect, Rapid diagnostic test (RDT)	N IgG (Abbott), N IgM (RDT), N IgG (RDT)	
Iriemenam	x-MAP multisntigen	N IgG, RBD IgG, S1 IgG	
Steinhardt	Euroimmun, Abbott Architect	N IgG (Euroimmun), N IgG (Abbott)	*Plasmodium* antibodies, *Plasmodium* antigens, antibodies for filariasis, oncocherciasis, syphilis/yaws, cystocercosis, taeniasis
Gelanew	ELISA	RBD IgG	
Gebrecherkos	Canea, Cellex, VivaChek,	N+S IgG or IgM (Cenea,	

	Innovita; ECLIA Roche	Cellex, VivaChek, Innovite), N all immunoglobulin (Roche)	
Mboumba Bouassa	BIOSYNEX, SIENNA, NG-test	RBD IgG and RBD IgM (BIOSYNEX), unknown antigen IgG and unknown antigen IgM (SIENNA), unknown antigen IgG and unknown antigen IgM (NG-test)	
Gdoura	Vidas Biomerieux, Elecsys Roche	RBD IgG (Vidas), N all subclasses (Elecsys)	
Vanroye	13 RDTs	N IgG or IgM (Toda, Biohit, Panbio, Boson), N+S IgG or IgM (Cellex, Dynamiker, Liming Bio), RBD IgG or IgM (ZenTech), N+RBD IgG or IgM (SureScreen Diagnostics, Singuway), N+RBD+S IgG or IgM (Multi-G), S IgG or IgM (Healgen), S (Wantai)	
Lapidus	ELISA	S1 IgG, S1 IgM	All participants have had malaria (different

			clinical phenotypes)

Table 3. Summary estimates of anti-SARS-CoV-2 antibody positivity in pre-pandemic African samples

GROUPS	Datasets	Positivity (95% CI), %	I^2
Antigenic target*			
Any N	38	15 (12-16)	96.8%
Any S	56	15 (13-17)	97.4%
S1	24	21 (16-25)	97.6%
RBD	15	9 (6-11)	97.0%
Any N +S	20	6 (3-9)	98.4%
Type of antibodies**			
IgG	82	13 (11-14)	96.9%
IgM	15	13 (8-18)	95.9%
IgG/IgM	16	14 (9-20)	97.0%
Malaria burden***			
High	95	17 (15-19)	97,5%
Low/None	22	1 (0-2)	69.2%
Dengue burden***			
High	92	15 (13-17)	97.0%
Low/None	25	11 (8-13)	97.0%
HIV burden***			
High	22	8 (6-10)	95.8%
Low/None	82	15 (13-16)	97.2%

*"Any S" includes S (n=9), S1 (n=24), S2 (n=4), RBD (n=15), RBM (n=3), NTD (n=1); "N+S" includes N+S (n=15), N+RBD (n=2), N+RBD+S (n=1) and N+S2 (n=2); for n=4 the antigen was unknown

**not shown are IgA (n=1 dataset) and all immunoglobulin subclasses (n=4 datasets)

***see Methods for definitions of subgroups

Supplementary Table 1: Joanna Briggs risk of bias assessment

	CRITERION	Scoring of studies
1	Was the sample representative of the target population?	Not applicable (no datasets had been collected with prior anticipation to be used for this pandemic-related question)
2	Were study participants recruited in an appropriate way?	Unclear in all studies
3	Was the sample size adequate?	Yes for Woodford, Baker, Souris, Gelanew and No for the other 18 studies (setting a threshold of having at least N=306 samples for 4% precision at 95% CI with expected proportion of positivity being 15%)
4	Were the study subjects and the setting described in detail?	No for Pedersen, Gelanew, and Gdoura and Yes for the other 15 studies if lenient about required information; most studies however did not give in-depth details
5	Was the data analysis conducted with sufficient coverage of the identified sample?	Yes for all studies, given that samples could be measured for antibodies with no/few missing measurements (although one cannot be certain of missingness at earlier stages of sampling)
6	Were objective, standard criteria used for the measurement of the condition?	Yes for all studies (based on providing definitions for positivity that are standard or defendable)
7	Was the condition measured reliably?	Unclear in all studies (it cannot be verified that antibody assays were performed reliably)
8	Was there appropriate statistical analysis?	Yes for all studies (if one requires only provision of

		positive and tested, not all studies gave confidence intervals, but these can be calculated in number of positives and of tested are given))
9	Are all important confounding factors/subgroups/differences identified and accounted for?	No for all studies, although several did explore some factors (as delineated in Table 2)
10	Were subpopulations identified using objective criteria?	Yes, for subpopulations listed in Table 2

Supplementary Table 2. Data on positivity and covariates of interest extracted

Author	Country	Antigenic targets	Antigen	Antibody	HIV high positivity	Malaria high burden	Dengue high burden	Positive	Tested
Pedersen	Gabon	S	S	IgG	No<10%	Yes	Yes	12	116
Pedersen	Gabon	N	N	IgG	No<10%	Yes	Yes	20	116
Pedersen	Senegal	S	S	IgG	No<10%	Yes	Yes	20	144
Pedersen	Senegal	N	N	IgG	No<10%	Yes	Yes	14	144
Borrega	Sierra Leone	N	N	IgG	No<10%	Yes	Yes	90	120
Borrega	Sierra Leone	RBD	RBD	IgG	No<10%	Yes	Yes	42	120
Borrega	Sierra Leone	S2	S2	IgG	No<10%	Yes	Yes	72	120
Tso	Tanzania	N	N	IgG	No<10%	No	Yes	18	105
Tso	Tanzania	S	S	IgG	No<10%	No	Yes	3	105
Tso	Zambia	N	N	IgG	Yes>10%	Yes	No	13	99
Tso	Zambia	S	S	IgG	Yes>10%	Yes	No	4	99
Emmerich	Magadascar	S1 Euroimmun	S1	IgG	No<10%	No	Yes	3	167 (0 b)
Emmerich	Magadascar	Nmod Euroimmun	N	IgG	No<10%	No	Yes	2	167 (0 b)
Emmerich	Magadascar	N EDI	N	IgG	No<10%	No	Yes	11	167 (10 b)
Emmerich	Magadascar	N Mikrogen	N	IgG	No<10%	No	Yes	1	167 (3 b)
Emmerich	Ghana 1	S1 Euroimmun	S1	IgG	No<10%	Yes	Yes	12	150 (1 b)
Emmerich	Ghana 1	Nmod Euroimmun	N	IgG	No<10%	Yes	Yes	14	150 (11 b)
Emmerich	Ghana 1	N EDI	N	IgG	No<10%	Yes	Yes	24	150 (17 b)
Emmerich	Ghana 1	N Mikrogen	N	IgG	No<10%	Yes	Yes	7	150 (7 b)
Emmerich	Ghana 2	S1 Euroimmun	S1	IgG	No<10%	Yes	Yes	4	133 (3 b)
Emmerich	Ghana 2	Nmod Euroimmun	N	IgG	No<10%	Yes	Yes	34	133 (21 b)
Emmerich	Ghana 2	N EDI	N	IgG	No<10%	Yes	Yes	39	133 (15 b)
Emmerich	Ghana 2	N Mikrogen	N	IgG	No<10%	Yes	Yes	22	133 (10 b)
Emmerich	Nigeria	S1 Euroimmun	S1	IgG	No<10%	Yes	Yes	14	150 (7 b)
Emmerich	Nigeria	Nmod Euroimmun	N	IgG	No<10%	Yes	Yes	42	150 (18 b)
Emmerich	Nigeria	N EDI	N	IgG	No<10%	Yes	Yes	91	150 (19 b)
Emmerich	Nigeria	N Mikrogen	N	IgG	No<10%	Yes	Yes	26	150 (6 b)
Yadouleton	Benin	S1 (Euroimmun)	S1	IgG	No<10%	Yes	No	8	60 (1b)
Yadouleton	Benin	S1 IgA (Euroimmun)	S1	IgA	No<10%	Yes	No	7	60
Yadouleton	Benin	S1 (InBios)	S1	IgG	No<10%	Yes	No	1	60
Yadouleton	Benin	N (Euroimmun)	N	IgG	No<10%	Yes	No	8	60 (1b)
Nzoghe	Gabon	N all subclasses	N	All Ig	No<10%	Yes	Yes	32	135
Woodford	Mali	S	S	IgG	No<10%	Yes	Yes	21	312
Woodford	Mali	N	N	IgG	No<10%	Yes	Yes	116	233
Woodford	Mali	RBD	RBD	IgG	No<10%	Yes	Yes	73	312

Baker	Uganda	Spike RBD IgM	RBD	IgM	No<10%	Yes	No	31	1077
Baker	Uganda	Spike RBD IgG	RBD	IgG	No<10%	Yes	No	7	1077
Gouris	DR Congo 1+2	S1	S1	IgG	No<10%	Yes	Yes	110	574
Gouris	DR Congo 1+2	S2	S2	IgG	No<10%	Yes	Yes	45	574
Gouris	DR Congo 1+2	S1-RBD	RBD	IgG	No<10%	Yes	Yes	33	574
Gouris	DR Congo 1+2	S1-NTD	NTD	IgG	No<10%	Yes	Yes	32	574
Gouris	DR Congo 1+2	N	N	IgG	No<10%	Yes	Yes	42	574
Gouris	Cameroon	S1	S1	IgG	Yes>10%	Yes	Yes	124	383
Gouris	Cameroon	S2	S2	IgG	Yes>10%	Yes	Yes	69	383
Gouris	Cameroon	S1-RBD	RBD	IgG	Yes>10%	Yes	Yes	85	383
Gouris	Cameroon	S1-NTD	NTD	IgG	Yes>10%	Yes	Yes	23	383
Gouris	Cameroon	N	N	IgG	Yes>10%	Yes	Yes	22	383
Gouris	R Congo	S1	S1	IgG	No<10%	Yes	Yes	64	384
Gouris	R Congo	S2	S2	IgG	No<10%	Yes	Yes	31	384
Gouris	R Congo	S1-RBD	RBD	IgG	No<10%	Yes	Yes	28	384
Gouris	R Congo	S1-NTD	NTD	IgG	No<10%	Yes	Yes	19	384
Gouris	R Congo	N	N	IgG	No<10%	Yes	Yes	29	384
ge	Nigeria	S1 Abbott	S1	IgG	Yes>10%	Yes	Yes	0	100
ge	Nigeria	N Eurommun	N	IgG	Yes>10%	Yes	Yes	3	100
ge	Nigeria	S Eurommun	S	IgG	Yes>10%	Yes	Yes	0	100
ge	Nigeria	N or S2 Mologic	N+S2	IgG	Yes>10%	Yes	Yes	16	100
Traore	Mali	S	S	IgG	No<10%	Yes	Yes	62	283
Traore	Mali	RBD	RBD	IgG	No<10%	Yes	Yes	19	283
Traore	Mali	RBM	RBM	IgG	No<10%	Yes	Yes	25	283
ngoba	R Congo	Virotech	N	IgG	No<10%	Yes	Yes	3	82
Diagne	Senegal	N or S2	N+S2	IgG	No<10%	Yes	Yes	88	272
Diagne	Senegal	N	N	IgG	No<10%	Yes	Yes	32	152
Yansouni	Senegal	N (Abbott Architect)	N	IgG	No<10%	Yes	Yes	8	90
Yansouni	Senegal	N IgM (Standard Q-SD)	N	IgM	No<10%	Yes	Yes	9	100
Yansouni	Senegal	N IgG (Standard Q-SD)	N	IgG	No<10%	Yes	Yes	1	100
riemenam	Nigeria	N	N	IgG	Yes>10%	Yes	Yes	35	213
riemenam	Nigeria	RBD	RBD	IgG	Yes>10%	Yes	Yes	10	213
riemenam	Nigeria	S1	S1	IgG	Yes>10%	Yes	Yes	1	213
Steinhardt	Nigeria	N (Euroimmun)	N	IgG	Yes>10%	Yes	Yes	38	213 (7b)
Steinhardt	Nigeria	N (Abbott)	N	IgG	Yes>10%	Yes	Yes	13	212
Gelanew	Ethiopia	RBD	RBD	IgG	No<10%	No	Yes	30	365

Gebrecherkos	Ethiopia	LFIA-Canea, (N and S)	N+S	IgM	No<10%	No	Yes	0	50
Gebrecherkos	Ethiopia	LFIA-Canea, (N and S)	N+S	IgG	No<10%	No	Yes	0	50
Gebrecherkos	Ethiopia	LFIA-Canea, (N and S)	N+S	IgG/IgM	No<10%	No	Yes	0	50
Gebrecherkos	Ethiopia	LFIA Cellex (N and S)	N+S	IgM	No<10%	No	Yes	0	50
Gebrecherkos	Ethiopia	LFIA Cellex (N and S)	N+S	IgG	No<10%	No	Yes	0	50
Gebrecherkos	Ethiopia	LFIA Cellex (N and S) (N and S)	N+S	IgG/IgM	No<10%	No	Yes	0	50
Gebrecherkos	Ethiopia	LFIA-VivaCheck (N and S)	N+S	IgM	No<10%	No	Yes	0	50
Gebrecherkos	Ethiopia	LFIA-VivaCheck (N and S)	N+S	IgG	No<10%	No	Yes	0	50
Gebrecherkos	Ethiopia	LFIA-VivaCheck (N and S)	N+S	IgG/IgM	No<10%	No	Yes	0	50
Gebrecherkos	Ethiopia	LFIA-Innovita (N and S)	N+S	IgM	No<10%	No	Yes	2	50
Gebrecherkos	Ethiopia	LFIA-Innovita (N and S)	N+S	IgG	No<10%	No	Yes	0	50
Gebrecherkos	Ethiopia	LFIA-Innovita (N and S)	N+S	IgG/IgM	No<10%	No	Yes	2	50
Gebrecherkos	Ethiopia	ECLIA Roche (N)	N	All Ig	No<10%	No	Yes	0	50
Mboumba Bouassa	Central Africa Republic	BIOSYNEX IgG	RBD	IgG	Yes>10%	Yes	No	0	100
Mboumba Bouassa	Central Africa Republic	BIOSYNEX IgM	RBD	IgM	Yes>10%	Yes	No	3	100
Mboumba Bouassa	Central Africa Republic	SIENNA IgG	Unknown	IgG	Yes>10%	Yes	No	0	100
Mboumba Bouassa	Central Africa Republic	SIENNA IgM	Unknown	IgM	Yes>10%	Yes	No	8	100
Mboumba Bouassa	Central Africa Republic	NG-test IgG	Unknown	IgG	Yes>10%	Yes	No	1	95
Mboumba Bouassa	Central Africa Republic	NG-test IgM	Unknown	IgM	Yes>10%	Yes	No	9	95
Vanroye*	Diverse countries	Toda IgG/IgM (N)	N	IgG/IgM	Unknown	Yes	No	6	195
Vanroye*	Diverse countries	Cellex IgG/IgM (N+S)	N+S	IgG/IgM	Unknown	Yes	No	7	195
Vanroye*	Diverse countries	Mutli-G IgG/IgM	N+RBD	IgG/IgM	Unknown	Yes	No	13	195

		(N+RBD+S)	+S						
Vanroye*	Diverse countries	Sure Screen IgG/IgM (N+RBD)	N+RBD	IgG/IgM	Unknown	Yes	No	14	195
Vanroye*	Diverse countries	Strong Strep IgG/IgM (N+S)	N+S	IgG/IgM	Unknown	Yes	No	17	195
Vanroye*	Diverse countries	QuickZen IgG/IgM (RBD)	RBD	IgG/IgM	Unknown	Yes	No	24	195
Vanroye*	Diverse countries	Biohit IgG/IgM (N)	N	IgG/IgM	Unknown	Yes	No	31	195
Vanroye*	Diverse countries	Singuway IgG/IgM (N+RBD)	N+RBD	IgG/IgM	Unknown	Yes	No	41	195
Vanroye*	Diverse countries	Panbio IgG/IgM (N)	N	IgG/IgM	Unknown	Yes	No	44	195
Vanroye*	Diverse countries	Dynamiker IgG/IgM (N+S)	N+S	IgG/IgM	Unknown	Yes	No	57	195
Vanroye*	Diverse countries	Healgen IgG/IgM (S)	S	IgG/IgM	Unknown	Yes	No	96	195
Vanroye*	Diverse countries	Wanti (ND) (S)	S	Unclear	Unknown	Yes	No	78	195
Vanroye*	Diverse countries	Boson IgG/IgM (N)	N	IgG/IgM	Unknown	Yes	No	100	195
Lapidus	Cameroon	S1	S1	IgG	No<10%	Yes	Yes	4	19
Lapidus	Cameroon	S1	S1	IgM	No<10%	Yes	Yes	2	19
Lapidus	Senegal1	S1	S1	IgG	No<10%	Yes	Yes	53	120
Lapidus	Senegal1	S1	S1	IgM	No<10%	Yes	Yes	40	120
Lapidus	Senegal2	S1	S1	IgG	No<10%	Yes	Yes	45	67
Lapidus	Senegal2	S1	S1	IgM	No<10%	Yes	Yes	56	67
Lapidus	BurkinaFasso1	S1	S1	IgG	No<10%	Yes	Yes	21	88
Lapidus	BurkinaFasso1	S1	S1	IgM	No<10%	Yes	Yes	10	88
Lapidus	BurkinaFasso2	S1	S1	IgG	No<10%	Yes	Yes	10	25
Lapidus	BurkinaFasso2	S1	S1	IgM	No<10%	Yes	Yes	12	25
Lapidus	Ghana	S1	S1	IgG	No<10%	Yes	Yes	37	45
Lapidus	Ghana	S1	S1	IgM	No<10%	Yes	Yes	21	45
Gdoura	Tunisia	RBD	RBD	IgG	No<10%	No	No	0	116
Gdoura	Tunisia	N	N	IgG	No<10%	No	No	0	116

Supplementary Table 3. Data on paired IgM and IgG measurements on the same samples with the same assay

AUTHOR	COUNTRY	IgM only	IgG only	Both	None
Baker	Uganda	31	5	2	1039
Gebrecherkos	Ethiopia 1	0	0	0	50
Gebrecherkos	Ethiopia 2	0	0	0	50
Gebrecherkos	Ethiopia 3	0	0	0	50
Gebrecherkos	Ethiopia 4	2	0	0	48
Mboumpa Bouassa	Central Afr Rep 1	3	0	0	97
Mboumpa Bouassa	Central Afr Rep 2	8	0	0	92
Lapidus	Cameroon	0	2	2	15
Lapidus	Senegal 1	7	20	33	60
Lapidus	Senegal 2	17	6	39	5
Lapidus	Burkina Fasso 1	3	14	7	64
Lapidus	Burkina Fasso 2	5	3	7	10
Lapidus	Ghana	3	19	18	5

Supplementary Table 4. Data on *Plasmodium* parasitemia (Par) and anti-SARS-CoV-2 antibodies (Ab)

AUTHOR	Ab+Par+	Ab-Par+	Ab+/Par-	Ab-Par-
Emmerich 1	9	46	5	90
Emmerich 2	6	49	6	89
Emmerich 3	12	43	12	83
Emmerich 4	1	54	6	89

Supplementary Table 5. Data on HIV status and anti-SARS-CoV-2 antibodies (Ab)

AUTHOR	Ab+HIV+	Ab-HIV+	Ab+HIV-	Ab-HIV-
Tso Tanzania	0	7	18	80
Tso Zambia	5	38	8	48
Baker Uganda	17	442	21	597

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