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Limited specificity of commercially available SARS-CoV-2 IgG ELISAs in serum samples of African origin

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Abstract

OBJECTIVES Specific serological tests are mandatory for reliable SARS-CoV-2 diagnostics and seroprevalence studies. Here, we assess the specificities of four commercially available SARS-CoV-2 IgG ELISAs in serum/plasma panels originating from Africa, South America, and Europe.

METHODS 882 serum/plasma samples collected from symptom-free donors before the COVID-19 pandemic in three African countries (Ghana, Madagascar, Nigeria), Colombia, and Germany were analysed with three nucleocapsid-based ELISAs (Euroimmun Anti-SARS-CoV-2-NCP IgG, EDI™ Novel Coronavirus COVID-19 IgG, Mikrogen *recom*Well SARS-CoV-2 IgG), one spike/S1-based ELISA (Euroimmun Anti-SARS-CoV-2 IgG), and in-house common cold CoV ELISAs.

RESULTS High specificity was confirmed for all SARS-CoV-2 IgG ELISAs for Madagascan (93.4–99.4%), Colombian (97.8–100.0%), and German (95.9–100.0%) samples. In contrast, specificity was much lower for the Ghanaian and Nigerian serum panels (Ghana: NCP-based assays 77.7–89.7%, spike/S1-based assay 94.3%; Nigeria: NCP-based assays 39.3–82.7%, spike/S1-based assay 90.7%). 15 of 600 African sera were concordantly classified as positive in both the NCP-based and the spike/S1-based Euroimmun ELISA, but did not inhibit spike/ACE2 binding in a surrogate virus neutralisation test. IgG antibodies elicited by previous infections with common cold CoVs were found in all sample panels, including those from Madagascar, Colombia, and Germany and thus do not inevitably hamper assay specificity. Nevertheless, high levels of IgG antibodies interacting with OC43 NCP were found in all 15 SARS-CoV-2 NCP/spike/S1 ELISA positive sera.

CONCLUSIONS Depending on the chosen antigen and assay protocol, SARS-CoV-2 IgG ELISA specificity may be significantly reduced in certain populations probably due to interference of immune responses to endemic pathogens like other viruses or parasites.

keywords SARS-CoV-2, seroepidemiologic studies, immunoglobulin G, Enzyme-Linked Immunosorbent Assay, specificity, Africa

Sustainable Development Goals (SDGs): Good Health and Well-Being

Introduction

In February and early March 2020, the first COVID-19 cases were reported from African countries with a high level of international contacts (Egypt, Algeria, Nigeria, Morocco, South Africa) [1]. Following these primary importation events, case numbers rapidly increased due to intra-continental and intra-national transmission as well as further importation mainly from Europe and Asia [1, 2]. As of February 14th 2021, 2,723,431 laboratory-confirmed SARS-CoV-2 infections and 68,294 deaths caused by COVID-19 have been reported from the WHO Africa region [3].

Nevertheless, the observed incidence rates are still well below the estimated numbers that were expected based on the socioeconomic challenges many African countries are facing [4] and the numbers observed in Europe and the Americas [3]. Although testing capacities vary widely between African countries, this observation is not solely based on underreporting, but probably reflects both the lower median age of the African population and a different immune status induced by contact with endemic pathogens [4]. Besides modulating COVID-19 morbidity and mortality rates, the latter may also influence performance data of SARS-CoV-2 serological tests; in particular assay specificity may be challenged by previous or current infections with other endemic pathogens [5–7]. Therefore, analyses of this parameter in different populations are an important prerequisite to ensure reliable diagnostic procedures and seroprevalence studies.

Up to now, a plethora of ELISA tests for detection of anti-SARS-Cov-2 antibodies has been developed and commercialised [8]. Usually, these assays employ one of the major immunogenic coronavirus proteins [9] as antigen, that is the nucleocapsid protein (NCP) or defined subdomains of the spike protein (e.g. S1 or the isolated receptor binding domain (RBD)). In this study, we investigate the specificity of three NCP-based SARS-CoV-2 IgG ELISA kits and one spike/S1-based kit in pre-COVID-19 serum/plasma panels from three African countries (Ghana, Madagascar, Nigeria) as well as from South America (Colombia) and Europe (Germany).

Methods

Human serum/plasma samples

The study was performed using stored human serum/-plasma samples collected before the COVID-19 pandemic (Table 1). Collection of samples was approved by

Table 1 Sample panels used in the study

Serum panel	Ghana 1	Ghana 2	Madagascar	Nigeria	Colombia	Germany
Number	150	133	167	150	134	148
Sampling year	2014–2015	1999	2010	2018	2014	2004–2015
Sample type	Serum	Serum	Plasma	Serum	Serum	Serum
Donors	Children	Teens, adults	Pregnant women	Adults	Adults	Adults
Age (median, IQR)	6 (3–7)	22 (16–45)*	23 (20–30)	41 (30–58)†	27 (23–36)	39 (28–48)‡
Sex m/f (*n* (%))	72/78 (48/52)	61/72 (46/54)	0/167 (0/100)	71/79 (47/53)	55/79 (41/59)	79/35 (69/31)‡
Sampling site	Agogo	Villages in the central region of Ghana	Coastal (n = 99) and highland (n = 68) regions	Irrua	Valledupar	Hamburg
Plasmodium spp. Positive (*n* (%))	55 (37.7)§	Not tested	4 (2.4)§	Not tested	Not tested; no previous infections reported	Not tested
SD Bioline Dengue Duo Rapid Test IgG pos (*n* (%))	Not tested	Not tested	Not tested	Not tested	29 (21.6)	Not tested

f, female; IQR, interquartile range; M, male.
*n = 89 (exact age information not available for 43 donors (all 43: age ≥ 18 years))
†n = 149 (age information not available for one donor)
‡n = 114 (sex and exact age information not available for 34 donors (all 34: age ≥ 18 years))
§Parasitaemic samples were identified by microscopy (Ghana) and *Plasmodium*-specific RT-PCR (Madagascar), respectively.

the Ethics Committees of the Kwame Nkrumah University of Science and Technology (Kumasi/Ghana; CHRPE/AP/427/13, 2013), the Comité d'ethique de la Vice Primature Chargée de la Santé Publique (Antananarivo/Madagaskar; no. 051-CE/MINSAN, 2009), the Irrua Specialist Teaching Hospital (Irrua/Nigeria; ISTH/HREC/20171019/28, 2017), the Hospital Rosario Pumarejo de Lopez (Valledupar/Colombia, 2013), and the Medical Association Hamburg/Germany (no. PV4608, 2013). All studies complied with the Declaration of Helsinki. Written informed consent was obtained from all individuals or, in case of minors, from parents or legal guardians before enrolment. Data privacy protection was guaranteed by anonymisation of samples.

Commercially available assays

SARS-CoV-2 IgG ELISAs (Euroimmun Anti-SARS-CoV-2-NCP IgG ELISA (Euroimmun, Germany), Euroimmun Anti-SARS-CoV-2 IgG ELISA (Euroimmun, Germany), EDI™ Novel Coronavirus COVID-19 IgG ELISA (Epitope Diagnostics, US), Mikrogen *recom*Well SARS-CoV-2 IgG ELISA (Mikrogen, Germany), Table 2), line blotting (Euroline Anti-SARS-CoV-2 Profile IgG line blot (Euroimmun)), and surrogate neutralisation assays (sVNT, Genscript, US) were performed and evaluated according to the manufacturers' instructions.

SARS-CoV-2 IgG immunofluorescence testing (IIFT)

Testing was performed as described previously [10] using SARS-CoV-2 infected Vero cells fixed with acetone/methanol.

In-house common cold CoVs ELISA

IgG antibodies were detected using a patented platform technology [11, 12]. Briefly, diluted patient serum/plasma samples were co-incubated together with a biotinylated recombinant antigen for 24 h at 4 °C in a microwell plate coated with a recombinant IgG immune complex specific capture molecule. Following a washing step, the bound IgG/antigen immune complexes were visualised by subsequent application of horseradish peroxidase (HRP)-labelled streptavidin and the colorimetric HRP substrate tetramethylbenzidine (TMB). After stopping the enzymatic reaction, the assay result was generated by measuring the optical density of the solution in the well at 450/620 nm.

As antigens, bacterially expressed, N-terminally truncated common cold CoV nucleoproteins comprising the protein's dimerisation domain were used (OC43 (AIL49389.1): NCPΔ258, HKU1 (AGT17773.1): NCPΔ256/S290F, NL63 (AFO70495.1): NCPΔ222, 229E (NP_073556.1): NCPΔ235). An assay cut-off of OD450-OD620 = 0.3 was determined by comparison of the ELISA results with the results obtained with a commercially available lineblot (Euroline Anti-SARS-CoV-2 Profile IgG, Euroimmun) for 32 German healthy blood donors. Index values (iv) were calculated by dividing the measured OD450-OD620 values by 0.3; samples were classified as negative (iv < 0.7), borderline ($0.7 \leq$ iv < 1.3), or positive (iv ≥ 1.3).

Table 2 Commercially available ELISA kits used in the study

	Euroimmun Anti-SARS-CoV-2-NCP-ELISA IgG	Euroimmun Anti-SARS-CoV-2-ELISA IgG	*EDI*™ Novel Coronavirus COVID-19 IgG ELISA kit	Mikrogen *recom*Well SARS-CoV-2 IgG
Manufacturer	Euroimmun AG Lübeck, Germany	Euroimmun AG Lübeck, Germany	Epitope Diagnostics, Inc. San Diego, US	Mikrogen GmbH Neuried, Germany
Status	CE-IVD	CE-IVD, FDA EUA	CE-IVD	CE-IVD
Antibody isotype	IgG	IgG	IgG	IgG
Test format	96 well microplate	96 well microplate	96 well microplate	96 well microplate
Test principle	indirect ELISA	indirect ELISA	indirect ELISA	indirect ELISA
Antigen	NCP (modified)	spike (S1 domain)	NCP	NCP
Sample dilution	1:101	1:101	1:101	1:101
Interpretation index value (iv)	negative: iv < 0.8 borderline; $0.8 \leq$ iv < 1.1 positive: iv ≥ 1.1	negative: iv < 0.8 borderline; $0.8 \leq$ iv < 1.1 positive: iv ≥ 1.1	negative: iv ≤ 0.9 borderline; 0.9 < iv < 1.1 positive: iv ≥ 1.1	negative: iv < 1.0 borderline; $1.0 \leq$ iv < 1.2 positive: iv ≥ 1.2

CE, Conformité Européenne; EUA, Emergency Use Authorisation; FDA, Food and Drug Administration; IFU, Instructions for Use; IVD, *in vitro* diagnostics; NCP, nucleocapsid protein.

Statistical analysis

Statistical analyses (calculation of 95% confidence intervals, Fisher's exact test) were performed using GraphPad Prism.

Results

High percentage of false positive SARS-CoV-2 IgG ELISA results in pre-COVID-19 serum samples from Ghana and Nigeria

To assess the specificities of commercially available SARS-CoV-2 IgG ELISA tests in sample panels of different origin, *a priori* SARS-CoV-2 IgG negative samples (Table 1) collected from symptom-free donors before 2019 in Africa (Ghana, Madagascar, Nigeria), South America (Colombia) and Europe (Germany) were analysed with the Euroimmun Anti-SARS-CoV-2-NCP IgG ELISA (Euroimmun, Germany), the Euroimmun Anti-SARS-CoV-2 IgG ELISA (Euroimmun, Germany), the EDI™ Novel Coronavirus COVID-19 IgG ELISA (Epitope Diagnostics, US), and the Mikrogen *recom*Well SARS-CoV-2 IgG ELISA (Mikrogen, Germany) (Table 2). While IgG ELISA specificities where good to excellent for pre-COVID-19 samples originating from Colombia, Madagascar, and Germany, increased false positive rates were observed in *a priori* SARS-CoV-2 IgG negative sera from Ghana and Nigeria (Figure 1, Table 3).

Correlation of NCP- and spike/S1-based ELISA results

The index values obtained with the three assays employing the same antigen (recombinant NCP) showed a clear correlation (Figure 2). In contrast, only a small number of 15 out of 600 African samples (Ghana 1: 4/150, Ghana 2: 3/133, Madagascar: 0/167, Nigeria: 8/150) were concordantly classified as positive by both the NCP-based and the spike/S1-based Euroimmun IgG ELISA (Figure 3a–c), a criterion that is fulfilled by the vast majority of sera from PCR-confirmed COVID-19 patients in the convalescent phase [13]. Positive or borderline serum reactivity with recombinant SARS-CoV-2 NCP (respectively spike/S1) was confirmed for 5/7 (7/7) Ghanaian and 6/8 (7/8) Nigerian samples using a commercially available line blot (Euroline Anti-SARS-CoV-2 Profile IgG line blot (Euroimmun)) (Figure 3d). In addition, antibodies binding to SARS-CoV-2 spike S2 domain were found in 7/7 Ghanaian and 4/8 Nigerian samples (Figure 3a–d). However, all 15 samples tested negative in IgG IIFT using SARS-CoV-2-infected Vero cells [10] and showed no or only very weak activity in a SARS-CoV-2 surrogate virus neutralising test (sVNT, Genscript, US) (Figure 3d).

Assessment of antibodies directed against common cold CoVs

To investigate the influence of previous infections with common cold CoVs on SARS-CoV-2 ELISA specificity, the complete sample panel (n = 882) was assayed for IgG antibodies interacting with the C-terminal dimerisation domains of the OC43, HKU1, NL63, and 229E NCPs using an in-house ELISA. In concordance with the worldwide occurrence of these viruses, considerable fractions of all serum panels, including the ones from Germany and Colombia, reacted positive in these tests (Figure 4).

In addition, the 15 African sera concordantly classified as positive by both the NCP-based and the spike/S1-based Euroimmun ELISA (Figure 3a–c) were also analysed with the Euroline Anti-SARS-CoV-2 Profile IgG line blot (Euroimmun) (Figure 3d). All of them showed a strong signal with OC43 NCP (mean index value 5.6) that by far exceeded the signals detected for the NCPs of HKU1 (mean index value 0.68), NL63 (mean index value 1.2), 229E (mean index value 1.0), and also SARS-CoV-2 (mean index value 1.3). Strikingly, only 2/7 Ghanaian samples but 7/8 Nigerian samples reacted positive in the in-house OC43 ELISA employing an N-terminally truncated OC43 NCP as antigen (Figure 3d).

Influence of Plasmodium parasitaemia on ELISA specificity

Information about *Plasmodium* parasitaemia was only accessible for one of the Ghanaian panels (Ghana 1, 55/150 samples from symptom-free children with microscopically detectable parasitaemia) and the Madagascan (4/167 *Plasmodium*-PCR positive samples) panel. Here, a reduced specificity in Ghanaian parasitaemic *vs*. non-parasitaemic samples was observed for the Euroimmun Anti-SARS-CoV-2-NCP IgG ELISA but not for the other SARS-CoV-2 IgG ELISAs (Table 4).

Discussion

In our study, we report a markedly reduced specificity of four SARS-CoV-2 IgG serological assays in serum samples originating from countries of sub-Saharan Africa, that is Ghana and Nigeria. This observation is in concordance with recent reports from Benin [7], Malawi [14], Tanzania [6], and Zambia [6] describing performance data of commercially available ELISAs [7, 14] and IIFT using eukaryotic cells overexpressing SARS-CoV-2

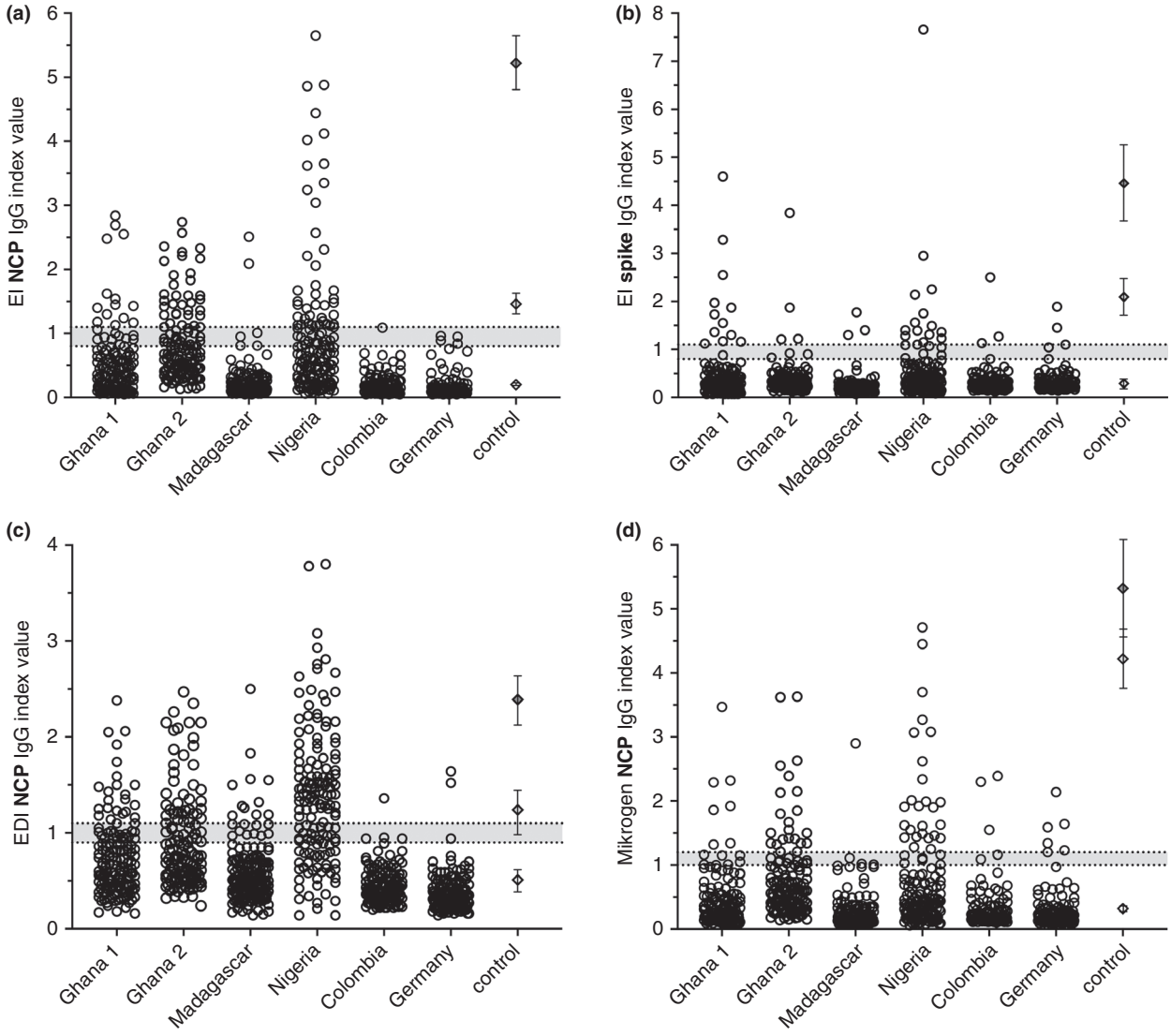


Figure 1 SARS-CoV-2 IgG ELISA results. (a–d) Index values obtained for serum/plasma samples collected before 2019 in three different African countries (Ghana panel 1 (n = 150), Ghana panel 2 (n = 133), Madagascar (n = 167), Nigeria (n = 150)), Colombia (n = 134), and Germany (n = 148) with the Euroimmun (EI) Anti-SARS-CoV-2-NCP IgG ELISA (a), the Euroimmun (EI) Anti-SARS-CoV-2 IgG ELISA (b), the EDI Novel Coronavirus COVID-19 IgG ELISA (c), and the Mikrogen *recom*Well SARS-CoV-2 IgG ELISA (d). Diamonds: index values obtained for two IgG positive COVID-19 patient sera sampled on day 19 post onset of symptoms (dark/-light grey: SARS-CoV-2 IgG IIFT titre 1:640/1:160) and one negative control serum; error bars: standard deviation of 13 (a, c) and 14 (b, d) independent measurements. Dotted lines: negative and positive cut-off values; grey shading indicates index values rated as 'borderline' according to the manufacturers' instructions.

proteins [6], respectively. Positive reactivity with both SARS-CoV-2 NCP and spike/S1 detected by ELISA could be confirmed by line blotting for 11/15 and 14/15 African sera, respectively. Thus, the line blot is slightly less sensitive in picking up the apparently false positive signals in the pre-COVID-19 African sera. As has previously been recognised for serological tests aiming at detection of anti-SARS-CoV-1 antibodies [9], our study revealed a higher rate of false positive assay results in the NCP-based IgG ELISAs than in the spike/S1-based IgG ELISA. This finding may reflect the higher degree of sequence conservation between the immunodominant regions of

Table 3 SARS-CoV-2 IgG ELISA specificities

		Euroimmun Anti-SARS-CoV-2-NCP-ELISA IgG	Euroimmun Anti-SARS-CoV-2-ELISA IgG	*EDI*™ Novel Coronavirus COVID-19 IgG ELISA kit	Mikrogen *recom*Well SARS-CoV-2 IgG
Antigen		NCP (modified)	Spike S1 domain	NCP	NCP
Ghana 1	pos bl neg	14 11 125	12 1 137	24 17 109	7 7 136
(*n* = 150)	specificity (95% CI)	90.7 (84.8–94.5)	92.0 (86.4–95.5)	84.0 (77.2–89.1)	95.3 (90.5–97.9)
Ghana 2	pos bl neg	34 21 78	4 3 126	39 15 79	22 10 101
(*n* = 133)	specificity (95% CI)	74.4 (66.4–81.1)	97.0 (92.3–99.1)	70.7 (62.4–77.8)	83.5 (76.2–88.9)
Ghana all	pos bl neg	48 32 203	16 4 263	63 32 188	29 17 237
(*n* = 283)	specificity (95% CI)	83.0 (78.2–87.0)	94.3 (90.9–96.6)	77.7 (72.5–82.2)	89.7 (85.6–92.8)
Madagascar	pos bl neg	2 4 161	3 0 164	11 10 146	1 3 163
(*n* = 167)	specificity (95% CI)	98.8 (95.5–99.9)	98.2 (94.6–99.6)	93.4 (88.5–96.4)	99.4 (96.3–100.0)
Nigeria	pos bl neg	42 18 90	14 7 129	91 19 40	26 6 118
(*n* = 150)	specificity (95% CI)	72.0 (64.3–78.6)	90.7 (84.8–94.5)	39.3 (31.9–47.3)	82.7 (75.8–87.9)
Colombia	pos bl neg	0 1 133	3 1 130	1 3 130	3 2 129
(*n* = 134)	specificity (95% CI)	100.0 (96.6–100.0)	97.8 (93.3–99.5)	99.2 (95.5–100.0)	97.8 (93.3–99.5)
Germany	pos bl neg	0 4 144	2 3 143	2 1 145	6 0 142
(*n* = 148)	specificity (95% CI)	100.0 (97.0–100.0)	98.6 (94.9–99.9)	98.6 (94.9–99.9)	95.9 (91.2–98.3)

CI, confidence interval; pos/bl/neg, number of samples rated as positive (pos), borderline (bl), and negative (neg) by the respective test. For calculation of specificities, both negative and borderline results were classified as 'not positive'.
Cells indicating the number of samples tested positive, borderline, and negative with the respective assay were shaded dark grey, medium grey, and light grey, respectively.

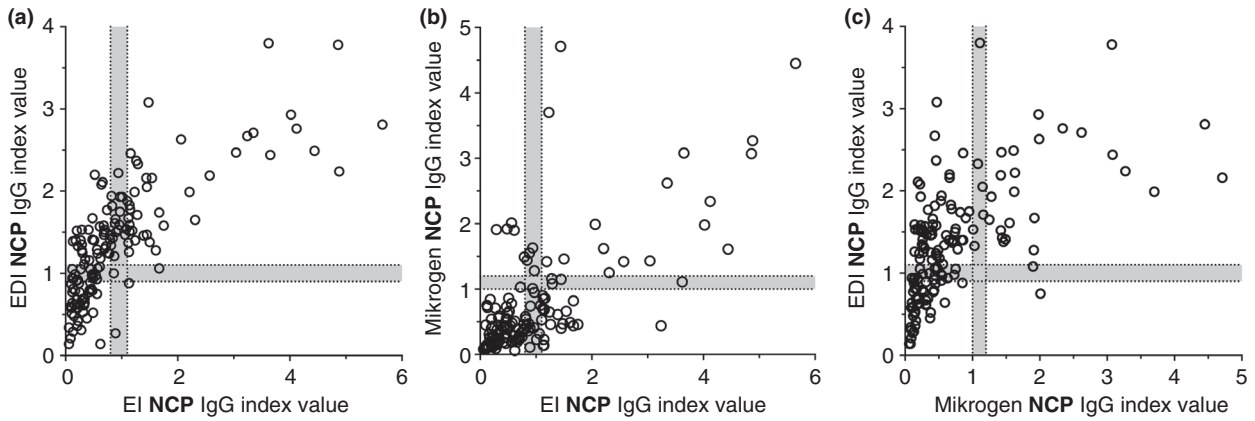
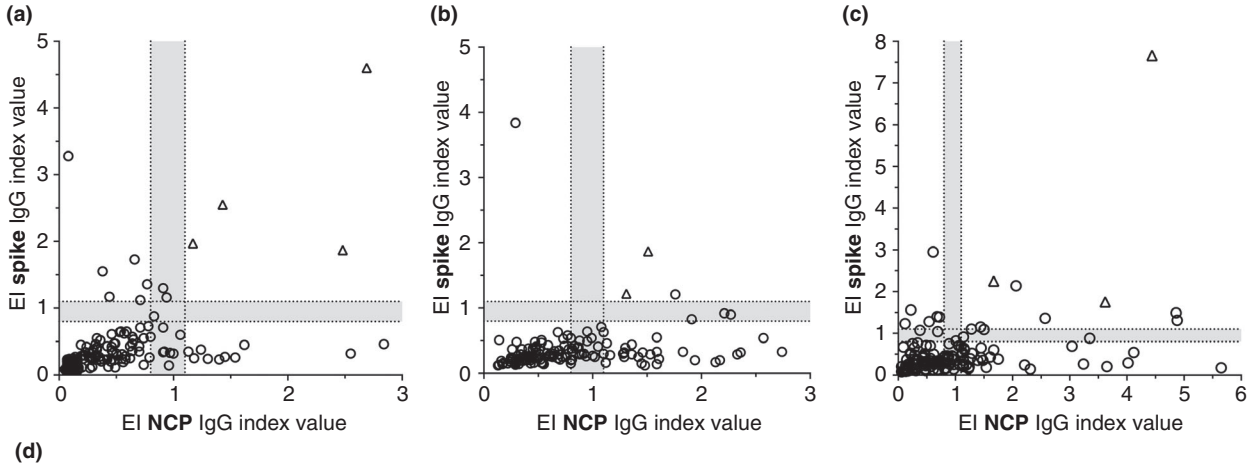


Figure 2 Correlation of SARS-CoV-2 IgG ELISA test results (NCP-based tests), exemplified for the Nigerian sample panel (*n* = 150). (a) Euroimmun (EI) Anti-SARS-CoV-2-NCP IgG ELISA vs. EDI Novel Coronavirus COVID-19 IgG ELISA, (b) Euroimmun (EI) Anti-SARS-CoV-2-NCP IgG ELISA *vs*. Mikrogen *recom*Well SARS-CoV-2 IgG ELISA, (c) Mikrogen *recom*Well SARS-CoV-2 IgG ELISA *vs*. EDI Novel Coronavirus COVID-19 IgG ELISA. Dotted lines: negative and positive cut-off values; grey shading indicates index values rated as 'borderline' according to the manufacturers' instructions.

the coronavirus NCPs [9], rendering assays based on this antigen more prone to cross-reactivity than tests employing the less conserved spike protein.

Indeed, one possible cause of the observed limited specificity of SARS-CoV-2 IgG ELISAs may be cross-reactivity with antibodies elicited by previous infections with other CoVs [6]. We detected such antibodies in considerable fractions of those sample panels for which excellent SARS-CoV-2 IgG ELISA specificity has been observed. Thus, their presence does not inevitably lead to



(d)

ID	sVNT % inh	Euroimmun ELISA		Euroimmun Lineblot							in-house ELISA			
		SARS-CoV-2		SARS-CoV-2			NCP				NCP-CTD			
		NCP	S1	NCP	S1	S2	OC43	HKU1	NL63	229E	OC43	HKU1	NL63	229E
G1-1	1.9	1.2	2.0	0.4	3.1	3.5	5.0	0.7	0.1	0.1	1.5	0.5	0.1	0.1
G1-2	9.4	2.7	4.6	0.9	5.3	5.2	6.2	0.5	0.4	0.2	0.4	0.3	0.2	0.2
G1-3	10.0	1.4	2.5	1.1	3.6	1.9	5.1	0.2	0.6	0.5	0.2	0.2	0.3	0.2
G1-4	4.1	2.5	1.9	0.5	1.6	2.4	4.1	0.9	2.8	1.2	2.9	0.3	10.1	0.2
G2-1	25.6	1.8	1.2	0.9	1.0	0.6	1.6	0.2	0.3	0.4	0.6	0.2	0.2	0.1
G2-2	24.6	1.5	1.9	0.9	1.7	2.2	7.1	0.4	2.1	0.9	0.2	0.3	2.0	0.1
G2-3	21.2	1.3	1.2	1.4	2.4	4.3	7.3	0.2	0.8	0.2	0.2	0.3	0.2	0.1
N-1	24.4	3.6	1.8	3.7	1.3	1.0	5.3	0.3	1.0	2.8	5.3	0.2	6.0	0.2
N-2	10.7	4.9	1.3	2.3	0.4	0.3	6.2	0.4	1.4	0.5	1.5	0.2	0.2	0.2
N-3	23.7	4.4	7.7	1.1	6.8	5.6	7.2	2.7	1.4	0.8	7.5	2.8	0.6	0.3
N-4	28.4	4.9	1.5	3.3	1.8	0.8	4.1	1.0	3.0	2.2	1.4	0.6	7.4	0.3
N-5	19.4	2.6	1.4	0.3	0.7	0.2	5.8	0.5	1.1	0.7	1.5	0.4	0.3	0.2
N-6	21.5	2.1	2.1	1.2	1.6	0.2	4.2	0.3	0.7	3.5	4.3	0.9	1.1	2.6
N-7	27.7	1.7	2.2	1.3	2.2	2.3	5.1	0.9	1.5	0.7	7.4	2.9	9.8	2.0
N-8	28.3	1.4	1.2	0.4	1.0	0.3	5.0	1.0	1.2	0.7	1.0	0.7	0.9	0.3

Figure 3 (a–c) Correlation of Euroimmun Anti-SARS-CoV-2-NCP IgG ELISA and Euroimmun Anti-SARS-CoV-2 IgG ELISA test results (NCP-based vs. spike/S1-based test). Shown are index values for sample panels Ghana 1 (a), Ghana 2 (b), and Nigeria (c). Triangles indicate spike/S1/NCP IgG positive samples containing IgG antibodies reacting positive with SARS-CoV-2 spike/S2 in the Euroimmun line blot. (D) ELISA, sVNT and line blot results for sera testing positive in both the Euroimmun NCP- and spike/S1-based IgG ELISA. Serum samples (Ghana 1 (G1): $n = 4$, Ghana 2 (G2): $n = 3$, Nigeria (N): $n = 8$)) were tested using the SARS-CoV-2 sVNT (Genscript) and the Euroline Anti-SARS-CoV-2 Profile IgG (Euroimmun) according to the manufacturer's instructions. Rating of index values (iv): Euroimmun ELISA: negative: iv < 0.8, borderline; 0.8 ≤ iv < 1.1, positive: iv ≥ 1.1; Line blot: negative: iv < 0.6, borderline: 0.6 ≤ iv < 1.0, positive: iv ≥ 1.0; in-house ELISA: negative: iv < 0.7, borderline: 0.7 ≤ iv < 1.3, positive: iv ≥ 1.3; sVNT: negative: % inhibition < 20.0, borderline; 20.0 ≤ % inh < 30.0, positive: % inh ≥ 30.0; CTD: C-terminal domain; dark grey fields: positive; light grey fields: borderline.

false positive signals. Nevertheless, the most challenging sample panel originating from Nigerian donors displayed the by far highest percentage of OC43 in-house-ELISA reactive samples (43.3%) and all 15 African samples (8 from Nigeria, 7 from Ghana) showing up false positive in both NCP- and spike/S1-based assays show extraordinarily high signals in an OC43 NCP line blot. Strikingly, all 8 Nigerian samples, but only 2/7 Ghanaian samples reacted with an N-terminally truncated, recombinant OC43 NCP in the in-house ELISA. This discrepancy between the line blot and the in-house ELISA results may be caused by (i) a lower sensitivity of the ELISA employing a truncated NCP displaying fewer epitopes as antigen (although an ELISA based on similar fragment of SARS-CoV-1 has been shown to detect anti-SARS-CoV-1 antibodies with excellent sensitivity [15]), (ii) a different conformational status of the used antigens (line blot: at least partially denatured, ELISA:

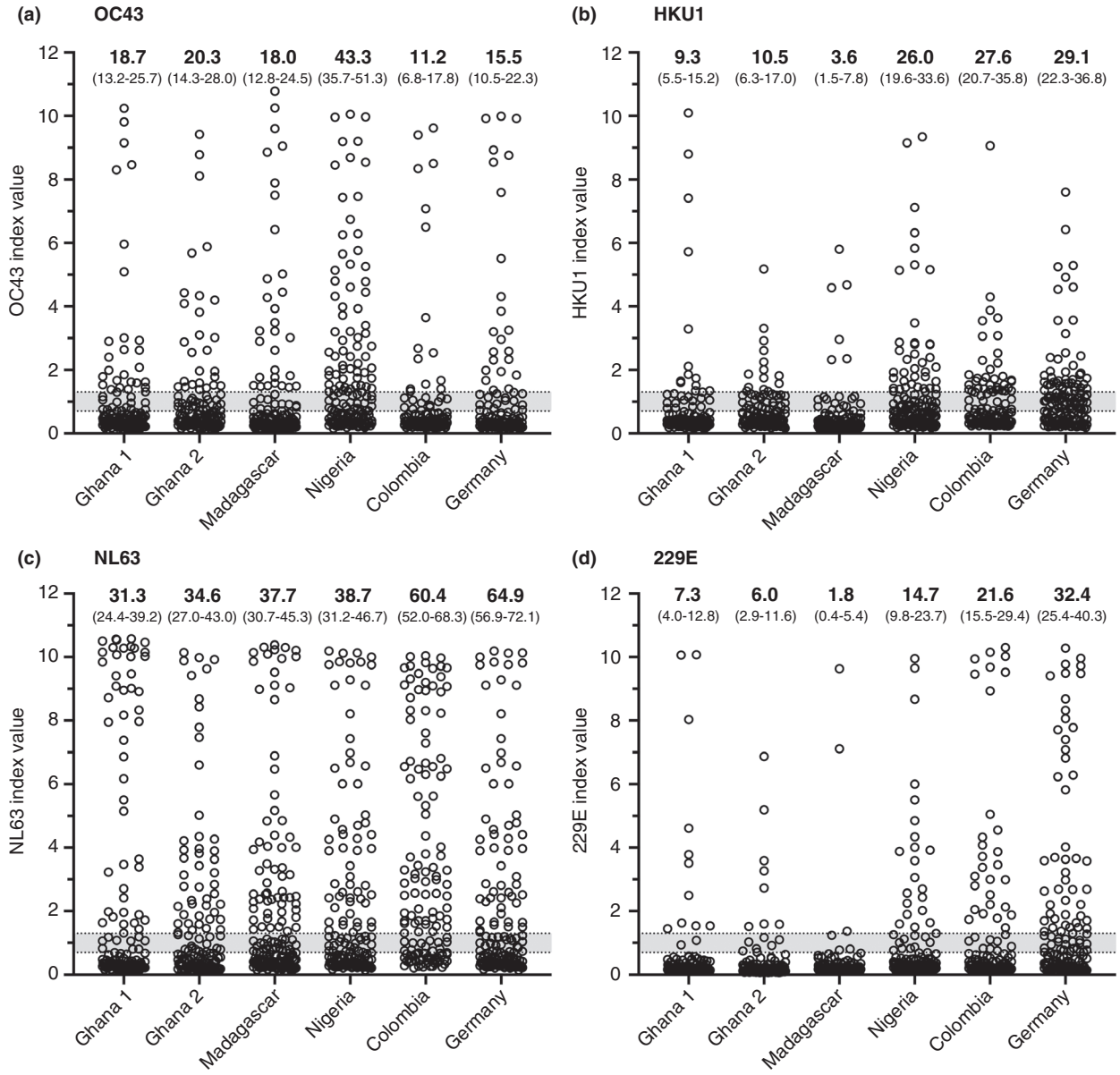


Figure 4 Common cold CoVs ELISA results. Index values obtained for the sample panels from Ghana (1: $n = 150$, 2: $n = 133$), Madagascar ($n = 167$), Nigeria ($n = 150$), Colombia ($n = 134$), and Germany ($n = 148$) using an in-house IgG ELISA protocol employing the C-terminal dimerisation domain of (a) OC43 NCP, (b) HKU1 NCP, (c) NL63 NCP, and (d) 229E NCP as antigen. Bold numbers: % of samples for which an iv ≥ 1.3 was obtained, numbers in brackets: 95% confidence interval. Grey shading indicates ivs rated as 'borderline' ($0.7 \leq$ iv < 1.3).

natively folded, soluble protein), (iii) blockage of epitopes by biotinylation of the ELISA antigen, (iv) different OC43 genotypes or closely related CoVs eliciting cross-reactive immune responses in the Nigerian and Ghanaian samples, respectively (this assumption is supported by the finding that 6/7 Ghanaian samples but only 4/8 Nigerian samples react borderline or positive with SARS-CoV-2 spike/S2 in the line blot). Taken together, at least some of the observed false positive signals could be due to previous infections with other coronaviruses sharing B cell epitopes with both OC43 and SARS-CoV-2.

Table 4 SARS-CoV-2 IgG ELISA specificities for donor subgroups from Ghana with and without *Plasmodium* parasitaemia (Ghana panel 1, symptom-free children)

				Euroimmun Anti-SARS-CoV-2-NCP-ELISA IgG			Euroimmun Anti-SARS-CoV-2-ELISA IgG			*EDI*™ Novel Coronavirus COVID-19 IgG ELISA kit			Mikrogen *recom*Well SARS-CoV-2 IgsG		
Parasitaemic (n = 55)	pos	bl	neg	9	6	40	6	1	48	12	9	34	1	4	50
	specificity (95% CI)			83.6 (71.5–91.4)			89.1 (77.8–95.3)			78.2 (65.5–87.2)			98.2 (89.5–100.0)		
Not parasitaemic (n = 95)	pos	bl	neg	5	5	85	6	0	89	12	8	75	6	3	86
	specificity (95% CI)			94.7 (88.0–98.0)			93.7 (86.6–97.3)			87.4 (79.1–92.8)			93.7 (86.6–97.3)		
P value				0.0387			0.3578			0.1675			0.4235		

pos/bl/neg: number of samples rated as positive (pos), borderline (bl), and negative (neg) by the respective test. CI: confidence interval. For calculation of specificities, both negative and borderline results were classified as 'not positive'. P values were calculated using Fisher's exact test (2 x 2 contingency table, pos versus 'not positive').
Cells indicating the number of samples tested positive, borderline, and negative with the respective assay were shaded dark grey, medium grey, and light grey, respectively.

As has already been reported for ZIKV [16] and SARS-CoV-2 IgG ELISAs [7], hypergammaglobulinaemia resulting from polyclonal B-cell activation induced by pathogens like *Plasmodia* can challenge assay specificity. Indeed, we observed a slightly reduced specificity of the Euroimmun Anti-SARS-CoV-2-NCP IgG ELISA in Ghanaian parasitaemic *vs*. non-parasitaemic samples (83.6% (95% CI: 71.5%-91.4%) *vs*. 94.7% (95% CI: 88.0%-98.0%), P = 0.0387)). While *Plasmodium falciparum* malaria is holoendemic in many regions in Ghana and Nigeria [17], length and intensity of malaria transmission varies significantly between different areas in Madagascar [17, 18]. In Colombia, 78% of the population lives in malaria free areas, and *Plasmodium falciparum* accounts only for 58% of malaria cases (42%: *Plasmodium vivax*)[19]. None of the 134 Colombian donors included in our study reported a previous malaria episode.

Recently, Lustig *et al.* [5] observed false positive results of spike-based IgA and IgG ELISAs in sera from donors with acute or past dengue virus infection. In the context of the study for which the samples were originally collected, 29 (21.6%) of the 134 Colombian donors had been tested positive in the SD Bioline Dengue Duo IgG Rapid Test (Alere/Abbott, USA), indicating a high titre of anti-DENV IgG antibodies [20]. Indeed, two of the three Colombian sera tested positive in the spike/S1-based Euroimmun Anti-SARS-CoV-2 ELISA belonged to this subgroup; a robust statistical evaluation of this finding would require significantly higher sample numbers.

Although antibodies binding to all three recombinant SARS-CoV-2 antigens presented on the Euroline Anti-SARS-CoV-2 Profile IgG line blot (NCP, spike/S1, spike/S2) were detected in some pre-COVID-19 African sera generating false positive ELISA results (Figure 3d), none of these samples showed up false positive in the in-house IgG IIFT using SARS-CoV-2 infected Vero cells. A possible explanation for this surprising observation might be the use of full virus displaying native proteins in their natural context vs. highly concentrated, purified, recombinant antigens. Indeed, increased numbers of false positive SARS-CoV-2 IgG IIFT results have been observed in African sera in a study using transfected HEK cells overexpressing SARS-CoV-2 antigens [6]. Furthermore, the specific SARS-CoV-2 staining pattern might be only generated when antibodies recognising different viral proteins with sufficiently high affinity are present in a tested serum. Interestingly, line blot intensity patterns for the different SARS-CoV-2 proteins (NCP, spike/S1, spike/S2) vary strongly between the 15 sera generating false positive signals in both the NCP and Spike IgG ELISA (Figure 3d).

Limitations of the study

Our study has some limitations that have to be acknowledged when interpreting the presented data. First of all, the sample panels (originating from previous studies with different scientific objectives) do not reflect representative cross-sections of the respective countries' populations and also comparability between sample panels is limited. Although in total 600 African samples have been analysed, the number of samples per country giving rise to false positive SARS-CoV-2 IgG assay results is still

relatively small, impeding statistical analyses. Furthermore, limited accessible sample volumes prevented us from performing material-intensive assays as the SD Bioline Dengue Duo IgG Rapid Test or *Plasmodium*-specific RT-PCR for the complete panel.

Conclusions

Our work shows that several commercially available SARS-CoV-2 IgG ELISAs, especially those employing recombinant NCP as antigen, are prone to generate a high number of false positive results when testing serum/-plasma samples originating from Sub-Saharan Africa. Beside other factors, high antibody titres resulting from previous infections with other coronaviruses and/or acute or previous malaria episodes may cause this phenomenon.

Based on these findings, the following recommendations should be considered when testing sera from African individuals or performing SARS-CoV-2 seroprevalence studies (not only) in Africa: (i) Carefully assess false positive signals obtained with the chosen serological test(s) in the target population (using *a priori* SARS-CoV-2 IgG negative serum samples which were stocked before 2019); if necessary adjust assay cut-off [14]. (ii) Be aware of potentially interfering/cross-reacting endemic pathogens and carefully interpret SARS-CoV-2 IgG test results in this context. (iii) If possible, combine information from two independent serological tests employing different antigens. In the context of the upcoming implementation of SARS-CoV-2 vaccine programmes, even other antigens than NCP and spike, for example ORF8 and ORF3b [21], might become relevant for differentiation of natural infections from vaccine responses although their use is limited due to high genetic variability [22, 23]. (iv) Re-evaluate samples generating a positive ELISA result by SARS-CoV-2 IgG IIFT and SARS-CoV-2 neutralisation testing.

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