Specificity of SARS-CoV-2 Antibody Detection Assays against S and N Proteins among Pre-COVID-19 Sera from Patients with Protozoan and Helminth Parasitic Infections

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ABSTRACT
We aimed to assess the specificity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody detection assays among people with tissue-borne parasitic infections. We tested three SARS-CoV-2 antibody-detection assays (cPass SARS-CoV-2 neutralization antibody detection kit [cPass], Abbott SARS-CoV-2 IgG assay [Abbott Architect], and Standard Q COVID-19 IgM/IgG combo rapid diagnostic test [SD RDT IgM/SD RDT IgG]) among 559 pre-COVID-19 seropositive sera for several parasitic infections. The specificity of assays was 95 to 98% overall. However, lower specificity was observed among sera from patients with protozoan infections of the reticuloendothelial system, such as human African trypanosomiasis (Abbott Architect; 88% [95% CI, 75 to 95]) and visceral leishmaniasis (SD RDT IgG; 80% [95% CI, 30 to 99]), and from patients with recent malaria in areas of Senegal where malaria is holoendemic (ranging from 91% for Abbott Architect and SD RDT IgM to 98 to 99% for cPass and SD RDT IgG). For specimens from patients with evidence of past or present helminth infection overall, test specificity estimates were all ≥96%. Sera collected from patients clinically suspected of parasitic infections that tested negative for these infections yielded a specificity of 98 to 100%. The majority (>85%) of false-positive results were positive by only one assay. The specificity of SARS-CoV-2 serological assays among sera from patients with protozoan and helminth parasitic infections was below the threshold required for decisions about individual patient care. Specificity is markedly increased by the use of confirmatory testing with a second assay. Finally, the SD RDT IgG proved similarly specific to laboratory-based assays and provides an option in low-resource settings when detection of anti-SARS-CoV-2 IgG is indicated.

KEYWORDS
SARS-CoV-2, COVID-19, diagnostic accuracy, antibody test, serology, parasitic infections, malaria, kinetoplastid infections, protozoan infections, helminth infections, Strongyloides, Schistosoma, filaria, Trichinella, neglected tropical diseases

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in the number and availability of serological assays detecting anti-SARS-CoV-2 antibodies, critical knowledge gaps remain regarding cross-reactivity of assays with sera from patients with noncoronavirus infectious agents.

In tropical regions of the world, several infections that dominate the local epidemiology of acute fever syndromes may cause nonspecific cross-reactivity with a wide range of serological assays (3–7). The mechanisms underlying cross-reactivity include infection of the reticuloendothelial system by several protozoans, with associated polyclonal B-lymphocyte proliferation (6, 7), and the broad diversity of antibodies elicited by various helminth infections (8). These infections include many neglected tropical diseases (NTDs) and malaria, for which the combined global burden exceeds 1.6 billion cases annually, with 3.8 billion at risk (9, 10). Simultaneously, three of the four countries with the largest total number of deaths attributed to COVID-19 are currently Brazil, India, and Mexico (Center for Systems Science and Engineering at Johns Hopkins University, COVID-19 dashboard, https://coronavirus.jhu.edu/map.html), all of which suffer a high burden of NTDs or malaria. As a result of this overlapping incidence, the specificity of SARS-CoV-2 serological tests may be different in countries where these infections are endemic compared to that in high-income countries. We aimed to assess the specificity of three SARS-CoV-2 antibody-detection assays against either the S or the N protein among a large collection of pre-COVID-19 sera from patients who were either ill with microbiologically proven malaria or seropositive for other tissue-borne parasitic infections.

MATERIALS AND METHODS

Ethics. This work was approved by the Research Ethics Boards of the Research Institute of the McGill University Health Centre (RI-MUHC number 2021-7246).

Sources of specimens tested. Specimens were well-characterized sera collected prior to July 2019 from patients with active or recent malaria imported to Canada, with active or recent malaria in an area where malaria is hyperendemic (Senegal), from clinical suspects for human African trypanosomiasis and for visceral leishmaniasis, from seropositives for Chagas disease, for Strongyloides sp., for Schistosoma sp., for filaria species, and for Trichinella sp. and from negative controls for whom tissue-borne parasitic infection was suspected but antibody testing was negative. The source and types of specimens are detailed in Table 1.
SARS-CoV-2 antibody testing. Three different SARS-CoV-2 antibody-detection assays were selected to assess the specificity of assays that detect different analytes, including anti-SARS-CoV-2 N-protein IgM, anti-SARS-CoV-2 N-protein IgG, and anti-receptor-binding domain (RBD) blocking antibodies of all immunoglobulin subclasses. We selected high-throughput assays for antibodies against N protein and RBD. In addition, among the three assays evaluated, we included an immunochromatographic rapid diagnostic test (RDT) that can be performed in low-resource settings and available from a quality-assured manufacturer with an international presence to enhance the relevance of this evaluation to the low-resource settings where NTDs and malaria are common. The RDT provides a separate readout for anti-N protein IgM and anti-N protein IgG, which we considered independently in our analysis.

Culture-free neutralization antibody detection assay (cPass). The cPass SARS-CoV-2 neutralization antibody detection kit (cPass) (Genscript, Piscataway, NJ) uses a blocking ELISA format with human ACE-2 receptor molecules coated on an ELISA plate (18, 21). Human sera preincubated with labeled epitopes of the RBD on S1 proteins are then transferred to the plate. This blocking ELISA serves as a surrogate assay to determine the capacity of human sera to block the interaction between the Spike fusion protein (through its RBD) and its cellular receptor ACE-2. The analyte detected is an anti-RBD blocking Ab of all subclasses. All the specimens, including positive and negative controls provided with the kit, were processed according to the manufacturer's instructions and included a 10-fold dilution factor of the primary specimen. All specimens and controls were tested in duplicate, and the percentage of inhibition calculation was based on the mean of OD for each duplicate. A cutoff 30% inhibition for SARS-CoV-2 neutralizing antibody detection was used to determine the presence of neutralizing antibodies, based on the manufacturer's instructions for use.

Abbott SARS-CoV-2 IgG assay (Abbott Architect). The Abbott SARS-CoV-2 IgG assay (Abbott Architect, Abbott Laboratories, Abbott Park, IL), which detects IgG against SARS-CoV-2 N protein, was performed on the Architect i2000sr platform according to the manufacturer's instructions. Specimens were thawed on the day of testing and were centrifuged at 10,000 × g for 10 min prior to each run. A sample-to-stored calibrator index (S/C) cutoff value of 1.4 was used for positive results, according to the manufacturer's recommendations.

Standard Q COVID-19 IgM/IgG Combo Rapid Test (SD RDT IgM and SD RDT IgG). The Standard Q COVID-19 IgM/IgG combo rapid test (SD BioSensor, Gyeonggi-do, Republic of Korea) is a rapid immunochromatographic diagnostic test (RDT) for the qualitative detection of specific IgM (SD RDT IgM) and IgG (SD RDT IgG) against SARS-CoV-2 N protein on two separate test lines. The RDT provides a separate readout for anti-N protein IgM and anti-N protein IgG, which we considered independently in our analysis. Serum specimens were processed according to the manufacturer's instructions. Briefly, 10 μl of serum were applied to the specimen well of the test device. Three drops (90 l) of buffer were added immediately and vertically into the same specimen well. The test results were read visually at within 15 min. According to the manufacturer, any visible band was considered a positive result. To facilitate analysis of positive test results, we further classified the intensity of test bands according to a standard color intensity scale provided by the manufacturer as follows: no signal (score of 0), barely visible but present (score of 1), low intensity (faint but definitively positive; score of 2), and medium to high intensity (score of 3) (Fig. 1).

Statistical analysis. Because all specimens were collected in the prepandemic era, prior to July 2019, all positive results for SARS-CoV-2 antibodies were considered false positives. The primary outcome calculated was test specificity and its corresponding 95% confidence intervals (95% CI), estimated according to a binomial distribution using the Wilson score method with Yates’s continuity correction as appropriate. The secondary outcome was relative risk (RR) for a false positive and the associated 95% CI. Both were estimated according to (i) positivity status for each parasite of interest and (ii) SARS-CoV-2 target antigen tested. Statistical analyses were performed using R version 3.5.2 (R Core Team, Vienna, Austria). Area-proportional Venn diagrams were generated using eulerAPE version 3.22.

RESULTS

Specificity of three commercial SARS-CoV-2 serological assays. The origin and characteristics of pre-COVID-19 specimens are reported in Table 1. Table 2 presents test specificity across the 559 samples tested. Overall, the point estimates of specificity of the cPass (10 of 559: 98%; 95% CI, 97 to 99) and SD rapid diagnostic test (RDT) IgG results (15 of 559: 97%; 95% CI, 96 to 98) were similar to those for Abbott Architect (26 of 548: 95%; 95% CI, 93 to 97) and SD RDT IgM result (30/559: 95%; 95% CI, 92 to 96).

For specimens from patients with evidence of blood- or tissue-invasive protozoan infections overall, test specificity was as follows: cPass (4 of 339: 99%; 95% CI, 97 to 99), SD RDT IgG (10 of 339: 97%; 95% CI, 95 to 98), Abbott Architect (19 of 328: 94%; 95% CI, 91 to 96), and SD RDT IgM results (23 of 339: 93%; 95% CI, 90 to 95). For specimens from Senegalese patients with malaria, specificity ranged from 91% (95% CI, 84 to 95) for the Abbott Architect and SD RDT IgM results to 99% (95% CI, 94 to 100) for the SD RDT IgG results. For specimens from travelers with imported malaria, test specificity ranged between 92 and 99%. Among sera positive for anti-Leishmania sp. and anti-Trypanosoma cruzi antibodies, cPass, Abbott Architect, and SD RDT IgM displayed 100% specificity. However, the SD RDT IgG result yielded specificities of 80% (95% CI, 30 to 99) and 92% (95% CI, 81 to 97) against visceral leishmaniasis and human American trypanosomiasis, respectively. The SD
RDT IgG result did not generate any false positives when used against human African trypanosomiasis specimens, whereas specificity of 88% (95% CI, 75 to 95) was observed for Abbott Architect.

For specimens from patients with evidence of past or present helminth infection overall, test specificity estimates were all ≥96%. When evaluated against specimens seropositive for *Strongyloides* sp. (n = 50) and *Trichinella* sp. (n = 30), specificities ranged from 96 to 98%. Test specificity assessed against specimens seropositive for filarial species (n = 40) ranged from 92 to 95% and from 92 to 97% against specimens seropositive for *Schistosoma* sp. (n = 40).

Sera collected from patients clinically suspected of parasitic infections that tested negative were also assessed. cPass yielded a specificity of 98% (1 false positive out of 60), whereas Abbott Architect, SD RDT IgG, and SD RDT IgM showed a specificity of 100%.

To allow statistical comparisons across the entire group, we computed the relative risk (RR) and 95% CI of a false-positive result by assay and target analyte, according to specimen origin (Table 3). Compared to cPass, the risk of a false-positive SARS-CoV-2 result was higher for the Architect and the SD RDT IgM result overall across all specimens (RR, 2.65; 95% CI, 1.29 to 5.45; and RR, 3.00; 95% CI, 1.48 to 6.08, respectively), for malaria specimens overall (RR, 4.89; 95% CI, 1.42 to 16.79; and RR, 7.00; 95% CI, 2.11 to 23.16), and for protozoan infections overall (RR, 4.91; 95% CI, 1.69 to 14.28; and RR, 5.75; 95% CI, 2.01 to 16.45). No significant differences were seen across assays for helminthic infections. SD RDT IgG relative risk of false positive was not significantly different from that of cPass for any of the specimen origins.

Characterization of false-positive results in terms of categorical agreement and signal intensity across serological assays. Categorical agreement between commercial serological assays for the detection of SARS-CoV-2 is depicted in Fig. 2. The majority (>85%) of false-positive results were positive by only one of the assays tested. When comparing cPass, Abbott Architect, and SD RDT IgG (Fig. 2A); cPass, Abbott Architect, and SD RDT IgM
### TABLE 2 Diagnostic specificity of three commercial serological assays for detection of SARS-CoV-2

| Pre-COVID specimen origin | No. | Assay      | Analyte detected                               | FP | TN | Specificity (95% CI) (%) |
|---------------------------|-----|------------|------------------------------------------------|----|----|-------------------------|
| **Confirmed active or recent malaria by microscopy or RDT (Senegal, area where malaria is endemic)** | 100 | cPass*     | Anti-RBD$^b$ blocking Ab,$^g$ all subclasses | 2  | 98 | 98 (93 to 99)           |
| 90$^b$                    |     | Abbott Architect | Anti-N-IgG | 8  | 82 | 91 (83 to 95)           |
| 100                      |     | SD RDT IgM   | Anti-N-IgM | 9  | 91 | 91 (84 to 95)           |
| 100                      |     | SD RDT IgG   | Anti-N-IgG | 1  | 99 | 99 (94 to 100)          |
| **Patients in whom a separate whole blood specimen was positive for malaria by PCR within the same 14 days (NRCP, area where malaria is not endemic)** | 143 | cPass     | Anti-RBD blocking Ab, all subclasses | 1  | 142 | 99 (96 to 100)         |
| 142$^c$                   |     | Abbott Architect | Anti-N-IgG | 6  | 136| 96 (91 to 98)           |
| 143                      |     | SD RDT IgM   | Anti-N-IgM | 12 | 131| 92 (86 to 95)           |
| 143                      |     | SD RDT IgG   | Anti-N-IgG | 4  | 139| 97 (93 to 99)           |
| **Visceral leishmaniasis** | 5  | cPass     | Anti-RBD blocking Ab, all subclasses | 0  | 5  | 100 (46 to 100)         |
| 5                        |     | Abbott Architect | Anti-N-IgG | 0  | 5  | 100 (46 to 100)         |
| 5                        |     | SD RDT IgM   | Anti-N-IgM | 0  | 5  | 100 (46 to 100)         |
| 5                        |     | SD RDT IgG   | Anti-N-IgG | 1  | 4  | 80 (30 to 99)           |
| **Human African trypanosomiasis** | 42 | cPass     | Anti-RBD blocking Ab, all subclasses | 1  | 41 | 98 (88 to 99)           |
| 42                       |     | Abbott Architect | Anti-N-IgG | 5  | 37 | 88 (75 to 95)           |
| 42                       |     | SD RDT IgM   | Anti-N-IgM | 2  | 40 | 95 (84 to 99)           |
| 42                       |     | SD RDT IgG   | Anti-N-IgG | 0  | 42 | 100 (89 to 100)         |
| **T. cruzi seropositivity** | 49 | cPass     | Anti-RBD blocking Ab, all subclasses | 0  | 49 | 100 (91 to 100)         |
| 49                       |     | Abbott Architect | Anti-N-IgG | 0  | 49 | 100 (93 to 100)         |
| 49                       |     | SD RDT IgM   | Anti-N-IgM | 0  | 49 | 100 (93 to 100)         |
| 49                       |     | SD RDT IgG   | Anti-N-IgG | 4  | 45 | 92 (81 to 97)           |
| **Overall protozoan parasitic infections (malaria/leishmaniasis/trypanosomiasis)** | 339 | cPass | Anti-RBD blocking Ab, all subclasses | 4  | 335 | 99 (97 to 99)          |
| 328                      |     | Abbott Architect | Anti-N-IgG | 19 | 309| 94 (91 to 96)           |
| 339                      |     | SD RDT IgM   | Anti-N-IgM | 23 | 316| 93 (90 to 95)           |
| 339                      |     | SD RDT IgG   | Anti-N-IgG | 10 | 329| 97 (95 to 98)           |
| **S. stercoralis seropositivity** | 50 | cPass | Anti-RBD blocking Ab, all subclasses | 2  | 48 | 96 (86 to 99)           |
| 50                       |     | Abbott Architect | Anti-N-IgG | 1  | 49 | 98 (89 to 100)          |
| 50                       |     | SD RDT IgM   | Anti-N-IgM | 1  | 49 | 98 (89 to 100)          |
| 50                       |     | SD RDT IgG   | Anti-N-IgG | 1  | 49 | 98 (89 to 100)          |
| **Schistosoma sp. seropositivity** | 40 | cPass | Anti-RBD blocking Ab, all subclasses | 1  | 39 | 97 (87 to 99)           |
| 40                       |     | Abbott Architect | Anti-N-IgG | 2  | 38 | 95 (83 to 99)           |
| 40                       |     | SD RDT IgM   | Anti-N-IgM | 3  | 37 | 92 (80 to 97)           |
| 40                       |     | SD RDT IgG   | Anti-N-IgG | 1  | 39 | 97 (87 to 99)           |
| **Filaria sp. seropositivity** | 40 | cPass | Anti-RBD blocking Ab, all subclasses | 2  | 38 | 95 (83 to 99)           |
| 40                       |     | Abbott Architect | Anti-N-IgG | 3  | 37 | 92 (80 to 97)           |
| 40                       |     | SD RDT IgM   | Anti-N-IgM | 2  | 38 | 95 (83 to 99)           |
| 40                       |     | SD RDT IgG   | Anti-N-IgG | 2  | 38 | 95 (83 to 99)           |
| **Trichinellosis (Trichinella sp.)$^d$** | 30 | cPass | Anti-RBD blocking Ab, all subclasses | 0  | 30 | 100 (86 to 100)         |
| 30                       |     | Abbott Architect | Anti-N-IgG | 1  | 29 | 97 (83 to 99)           |
| 30                       |     | SD RDT IgM   | Anti-N-IgM | 1  | 29 | 97 (83 to 99)           |
| 30                       |     | SD RDT IgG   | Anti-N-IgG | 1  | 29 | 97 (83 to 99)           |

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or cPass, Abbott Architect, and SD RDT IgG or SD RDT IgM (Fig. 2C), all three assays were in agreement for only 14% (5 of 36), 7.8% (4 of 51), or 8.3% (5 of 60) of the false-positive results, respectively. When comparing SD RDT IgG and SD RDT IgM (Fig. 2D), the two assays were in agreement for 15% (6 of 39) of the specimens with a false-positive result.

Readout intensities of each serological test were assembled in a heat map for specimens with false-positive results from one or more tests (Fig. 3). Overall, among specimens with false-positive results, there was very little correlation between the signal intensity of a false-positive test result and the probability of a false positive with another assay. Strong signal intensities were common among false-positive results from laboratory-based assays. The cPass yielded positive results for 10 of 60 (16.7%) specimens with false-positive results from one or more tests, with 5 of these having a binding inhibition of $\geq 30\%$. The cutoff used to determine Abbott Architect positivity was a sample-to-stored calibrator index (S/C) of $>1.4$.

**DISCUSSION**

We sought to assess the specificity of three SARS-CoV-2 antibody detection assays among people who were either ill with microbiologically proven malaria or seropositive for other tissue-borne parasitic infections. We tested assays against either the S or the N protein, among a large collection of well-characterized pre-COVID-19 sera from clinical suspects with relevant tropical infectious diseases that may lead to cross-reactions with SARS-CoV-2 serologic assays. Previous reports found increased frequency of nonspecific binding leading to positive results in smaller cohorts of patients with and without recent malaria in Nigeria (11), Benin (12), and Tanzania and Zambia (13). We confirm these findings with different serological assays and extend them to patients with imported malaria residing in an area where malaria is not endemic, as well as to patients with several key tropical infectious diseases for which there is a...
TABLE 3  Relative risk of false-positive result by assay and target analyte, according to specimen origin

| Pre-COVID specimen origin | No. | Assay          | Analyte detected | FP   | TN   | Risk ratio (95% CI)         |
|---------------------------|-----|----------------|------------------|------|------|-----------------------------|
| **Confirmed active or recent malaria by microscopy or RDT (Senegal, area where malaria is endemic)** |     |                |                  |      |      |                            |
| Patients in whom a separate whole blood specimen was positive for malaria by PCR within the same 14 days (NRCP, area where malaria is not endemic) |     |                |                  |      |      |                            |
| Overall malaria (Senegal and NRCP) |     |                |                  |      |      |                            |
| Visceral leishmaniasis |     |                |                  |      |      |                            |
| Human African trypanosomiasis |     |                |                  |      |      |                            |
| T. cruzi seropositivity |     |                |                  |      |      |                            |
| Overall protozoan parasitic infections (malaria/leishmaniasis/trypanosomiasis) |     |                |                  |      |      |                            |
| S. stercoralis seropositivity |     |                |                  |      |      |                            |
| Schistosoma sp. seropositivity |     |                |                  |      |      |                            |
| Filaria sp. seropositivity |     |                |                  |      |      |                            |
| Trichinellosis (Trichinella sp.) |     |                |                  |      |      |                            |

(Continued on next page)
The observed specificity of all assays ranged from 95 to 98% in the overall group of specimens. However, those for the Abbott Architect (95% [95% CI, 93 to 97]) and the SD RDT IgM (95% [95% CI, 93 to 96]) fell short of the World Health Organization-recommended 97% benchmark for SARS-CoV-2 serological assays (14). Moreover, these values are well below estimates for the Abbott Architect from previous data among specimens from high-income countries, including 99.6% reported by the manufacturer using a panel of pre-COVID-19 specimens.

# TABLE 3 (Continued)

| Pre-COVID specimen origin | No. | Assay        | Analyte detected | FP  | TN  | Risk ratio (95% CI) |
|---------------------------|-----|-------------|------------------|-----|-----|-------------------|
| Overall helminth infections (strongyloidiasis/schistosomiasis/filariasis/trichinellosis) | 160 | Abbott Architect | Anti-N-IgG | 7   | 153 | 1.40 (0.45 to 4.32) |
|                           | 160 | SD RDT IgM   | Anti-N-IgM       | 7   | 153 | 1.40 (0.45 to 4.32) |
|                           | 160 | SD RDT IgG   | Anti-N-IgG       | 5   | 155 | 1.00 (0.29 to 3.39) |
|                           | 160 | cPass        | Anti-RBD blocking Ab, all subclasses | 5   | 155 | Ref. |
| Sera from parasite suspects negative for all above pathogens | 60  | Abbott Architect | Anti-N-IgG | 0   | 60  | Ref. |
|                           | 60  | SD RDT IgM   | Anti-N-IgM       | 0   | 60  | Ref. |
|                           | 60  | SD RDT IgG   | Anti-N-IgG | 0   | 60  | Ref. |
|                           | 60  | cPass        | Anti-RBD blocking Ab, all subclasses | 1   | 59  | Ref. |
| Overall (all samples)     | 548 | Abbott Architect | Anti-N-IgG | 26  | 522 | 2.65 (1.29 to 5.45) |
|                           | 559 | SD RDT IgM   | Anti-N-IgM | 30  | 529 | 3.00 (1.48 to 6.08) |
|                           | 559 | SD RDT IgG   | Anti-N-IgG       | 15  | 544 | 1.50 (0.68 to 3.31) |
|                           | 559 | cPass        | Anti-RBD blocking Ab, all subclasses | 10  | 549 | Ref. |

*These specimens were drawn from patients clinically suspected of active disease for the purpose of diagnosis, as opposed to screening of asymptomatic individuals.

*The cutoff used to determine cPass positivity was ≥30% inhibition. The cutoff used to determine Abbott Architect positivity was a sample-to-stored calibrator index (S/C) of >1.4.

*Ref., Reference.

The current void of available information on which to base interpretation of serological results for SARS-CoV-2.

**FIG 2** Venn diagram comparing false-positive results from cPass, Abbott Architect, SD RDT IgG, and SD RDT IgM serology from patients with protozoan and helminth parasites infections. (A to C) Overlap of cPass and Abbott Architect with SD RDT IgG, SD RDT IgM, or any positive SD RDT result, respectively. (D) Overlap of SD RDT IgG and SD RDT IgM. The numbers denote the number of false-positive specimens in each category. RDT, rapid diagnostic test.
FIG 3  Heat map of readout signal intensity of all false-positive specimens identified using three commercial serological assays for the detection of SARS-CoV-2. The cutoffs used to determine cPass positivity were as (Continued on next page)
or from patients positive for alternative respiratory pathogens \( n = 1,070 \) (15) and 99.6% reported in an independent evaluation of 1,099 pre-COVID-19 specimens (16). Similarly, the values for the SD RDT IgM are lower than the 100% specificity reported in the FDA serology test evaluation report for the Standard Q COVID-19 IgM/IgG combo rapid test (17).

As expected, the lowest observed specificities were seen among sera from patients with protozoan infections of the reticuloendothelial system, such as human African trypanosomiasis (Abbott Architect; 88% [95% CI, 75 to 95]) and visceral leishmaniasis (SD RDT IgG; 80% [95% CI, 30 to 99]), and from patients with recent malaria from an area of Senegal where malaria is holoendemic (ranging from 91% for Abbott Architect and SD RDT IgM to 98 to 99% for cPass and SD RDT IgG). Nonspecific cross-reaction among patients in areas where malaria is endemic may be potentiated by coinfections rather than from malaria infections alone. Alternatively, repeated infections with Plasmodium species rather than coinfections with other organisms may lead to a greater cross-reactivity. This is consistent with the association between false-positive SARS-CoV-2 results and the presence of anti-Plasmodium antibodies (11), as well as the relatively higher specificity observed in our cohort of patients with antibodies to tissue-invasive helminths. Specificities among sera positive for the presence of antibodies to T. cruzi ranged from 92% [95% CI, 81 to 97] (SD RDT IgG) to 100% [95% CI, 93 to 100] (all other assays). Taken as a whole, the observed specificities among the assays and specimens tested are likely adequate for serosurveys and epidemiologic tracking but below the threshold required for individual patient care decisions (1, 14).

In order to allow statistical comparisons between different SARS-CoV-2 diagnostic assays, we computed the relative risk of a false-positive result by diagnostic assay and target analyte, according to specimen origin (Table 3). The cPass showed the least variation across specimen origins and consistently had the highest specificity across groups. This assay was designed as a surrogate viral neutralization assay and measures the strength of inhibition of RBD binding to ACE-2 by host antibodies of any subclass. Perhaps surprisingly for a lateral flow immunochromatographic SARS-CoV-2 assay, the SD RDT IgG also showed very high performance across groups. Using cPass as the reference value, SD RDT IgG had a lower relative risk (RR) of a false-positive result than either SD RDT IgM or Abbott Architect. The latter two tests were statistically significantly more likely to yield false-positive results than the cPass for specimens with evidence of protozoan infections overall but not for specimens with evidence of tissue-invasive helminth infections. We previously showed that cPass has marginal advantages over anti-RBD IgG enzyme-linked immunosorbent assay (ELISA) (18). In this case, the SD RDT IgG detects anti-N IgG and performed comparably to a sophisticated surrogate viral neutralization assay. Moreover, it compared favorably to the laboratory-based Abbott Architect in this group of specimens of interest. This is relevant to low-resource tropical settings where central laboratory capacity frequently limits care of patients with fever syndromes (19).

The finding of very low categorical agreement between SARS-CoV-2 serological assays among specimens with a false-positive result is consistent with nonspecific binding between host antibodies and test antigens. This observation can be leveraged to design testing algorithms with increased specificity. In our specimen set, requiring a positive result from a second test among cPass, Abbott Architect, or SD RDT IgG would rule out the majority of false-positive results obtained after a first positive result (Fig. 2 and Table 4). Others have proposed an avidity assay using various concentrations of urea washes to prevent nonspecific binding (11), but this approach may not be suitable in low-resource settings, even when centralized laboratories exist.

**FIG 3 Legend (Continued)**

follows: negative was \(<20\%\) inhibition; indeterminate was 20 to \(<30\%\) inhibition; and positive was \(\geq30\%\) inhibition. The criteria used to determine Abbott Architect signal strength were as follows: negative was a signal-to-cutoff ratio of \(<1.0\); weak positive was a signal-to-cutoff ratio of 1.0 to 1.2; and strong positive was a signal-to-cutoff ratio of \(\geq1.2\). In this case, the cutoff refers to the sample-to-stored calibrator index \(S/C\) cutoff value of 1.4, and a signal-to-cutoff ratio of 1.2 corresponds to an actual readout of 1.4 \(\times\) 1.2 = 1.68. The categorization for SD RDT band intensity was as follows: a score of 0 indicates no signal; 1 indicates barely visible but present; 2 indicates low intensity (i.e., faint but definitively positive); and 3 indicates medium to high intensity. PCR, polymerase chain reaction; NRCP, National Reference Centre for Parasitology.
TABLE 4 Impact of performing sequential serologic assays on specificity for the detection of anti-SARS-CoV-2 antibodies

| Assay 1            | Single-test Specificity (95% CI) (%) | Assay 2            | Combined specificity (95% CI) (%) |
|--------------------|-------------------------------------|--------------------|-----------------------------------|
| cPass              | 99 (97 to 99)                       | Abbott Architect   | 100 (98 to 100)                   |
|                    |                                     | SD RDT IgG         | 100 (98 to 100)                   |
| Abbott Architect   | 94 (91 to 96)                       | cPass              | 100 (98 to 100)                   |
|                    |                                     | SD RDT IgG         | 99 (98 to 100)                    |
| SD RDT IgM<sup>d</sup> | 93 (90 to 95)                  | cPass              | 99 (98 to 100)                    |
|                    |                                     | Abbott Architect   | 99 (97 to 100)                    |
|                    |                                     | SD RDT IgG         | 99 (98 to 100)                    |
| SD RDT IgG<sup>d</sup> | 97 (95 to 98)                  | cPass              | 100 (98 to 100)                   |
|                    |                                     | Abbott Architect   | 99 (98 to 100)                    |

<sup>a</sup>The diagnostic specificity of combinations of commercial serological assays for the detection of anti-SARS-CoV-2 antibodies was determined among all specimens positive for protozoan parasitic infections (n = 339). The order of the assays performed is accounted for in the combined specificity, because Assay 2 is only applied as a confirmatory test to specimens with a positive result by Assay 1.

<sup>b</sup>Wilson score interval binomial 95% confidence intervals presented.

<sup>c</sup>For the computation of combined specificities, positive results from both assays are required to determine a false positive. The order of assays performed is accounted for in the combined specificity, because Assay 2 is only applied as a confirmatory test to specimens with a positive result by Assay 1.

<sup>d</sup>SD RDT IgM results are not considered as results from Assay 2 because using the presence of IgM antibodies to confirm the presence of specific IgG antibodies is not felt to be a meaningful use case.

Limitations of our study include the fact that the available volume of stored prepandemic specimens precluded the possibility of performing specific avidity testing or assessing for the presence of antibodies to seasonal coronaviruses that may have cross-reacted with the SARS-CoV-2 serological assays. However, a report from the United States found no false positives for Abbott Architect or SD RDT IgM/IgG among 21 patients with recent seasonal coronavirus infections: NL63 (n = 11), HKU1 (n = 7), and 229E (n = 3) (20). Moreover, the fact that our data recapitulate findings from previous studies in areas where malaria is endemic is reassuring regarding their robustness.

**Conclusions.** Among sera from patients with tissue-borne parasitic infections, the specificity of SARS-CoV-2 serological assays was below the threshold required for decisions about individual patient care. Specificity is markedly increased by the use of confirmatory testing with a second assay. Finally, the SD RDT IgG proved similarly specific to laboratory-based assays and provides an option in low-resource settings when detection of anti-SARS-CoV-2 IgG is indicated.

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Conflicts of interest.

J.P. reports grants from MedImmune, grants from Sanoﬁ Pasteur, grants and personal fees from Seegene, and grants and personal fees from AbbVie outside the submitted work. M.P.C. reports personal fees from GEn1E Lifesciences (as a member of the scientiﬁc advisory board) and from nplex biosciences (as a member of the scientiﬁc advisory board) outside the submitted work. He is the cofounder of Kanvas Biosciences and owns equity in the company. In addition, M.P.C. has patents pending for methods for detecting tissue damage, graft versus host disease, and infections using cell-free DNA proﬁling pending and for methods for assessing the severity and progression of SARS-CoV-2 infections using cell-free DNA. C.P.Y. reports being on an independent data monitoring committee for Medicago Inc. All other authors declare no conﬂicts of interest.

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