Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

International Journal of Infectious Diseases 112 (2021) 281–287

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Evaluation of the performance of 25 SARS-CoV-2 serological rapid diagnostic tests using a reference panel of plasma specimens at the Uganda Virus Research Institute

Tom Lutalo[a], Aminah Nalumansi[a], Denis Olara[a], John Kayiwa[a], Bernard Ogwang[a], Emmanuel Odwilo[a], Christine Watera[a], Stephen Balinandi[a], Jocelyn Kiconco[a], Joweria Nakaseegu[a], Jennifer Serwanga[a,b], Bernard Kikaire[a], Deogratius Ssemwanga[a,b], Brendah Abiko[a], Christopher Nsereko[c], Matthew Cotten[b], Joshua Buule[a], Julius Lutwama[a], Robert Downing[a], Pontiano Kaleebu[a,b,*]

[a] Uganda Virus Research Institute, PO Box 49, Plot 51–59, Nakiwogo Road, Entebbe, Uganda
[b] Medical Research Council/Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine, Uganda Research Unit, PO Box 49, Plot 1–59, Nakiwogo Road, Entebbe, Uganda
[c] Entebbe Regional Referral Hospital, PO Box 29, Kampala Road, Entebbe, Uganda

ARTICLE INFO

Article history:
Received 21 May 2021
Revised 10 September 2021
Accepted 13 September 2021

Keywords:
COVID-19
SARS-CoV-2
Rapid diagnostic test
antibody
serological reference panel
performance

ABSTRACT

Introduction: Serological testing is needed to better understand the epidemiology of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus. Rapid diagnostic tests (RDTs) have been developed to detect specific antibodies, IgM and IgG, to the virus. The performance of 25 of these RDTs was evaluated.

Methods: A serological reference panel of 50 positive and 100 negative plasma specimens was developed from SARS-CoV-2 PCR and antibody positive patients and pre-pandemic SARS-CoV-2-negative specimens collected in 2016. Test performance of the 25 RDTs was evaluated against this panel.

Results: A total of 10 RDTs had a sensitivity ≥98%, while 13 RDTs had a specificity ≥98% to anti-SARS-CoV-2 IgG antibodies. Four RDTs (Boson, MultiG, Standard Q, and VivaDiag) had both sensitivity and specificity ≥98% to anti-SARS-CoV-2 IgG antibodies. Only three RDTs had a sensitivity ≥98%, while 10 RDTs had a specificity ≥98% to anti-SARS-CoV-2 IgM antibodies. Three RDTs (Autobio, MultiG, and Standard Q) had sensitivity and specificity ≥98% to combined IgG/IgM. The RDTs that performed well also had perfect or almost perfect inter-reader agreement.

Conclusions: This evaluation identified three RDTs with a sensitivity and specificity to IgM/IgG antibodies of ≥98% with the potential for widespread antibody testing in Uganda.

1. Introduction

Coronavirus disease 2019 (COVID-19) was declared a pandemic by the World Health Organization (WHO) on March 11, 2020. Globally as of September 1, 2021, there have been nearly 218 million cases reported to the WHO with over 4.5 million deaths, while in Uganda 99 408 cases with over 3000 deaths have been recorded (WHO, 2021). Since this evaluation, the number of new cases and deaths has continued to rise.

Standard laboratory confirmation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is based on the detection of unique viral sequences in nasopharyngeal samples by nucleic acid amplification test (NAAT) (WHO, 2020b). Although the priority intervention from a public health perspective has been to identify those with acute infection and to quarantine them and their immediate contacts in order to control the spread of infection, it has become apparent that it is also important to identify convalescent cases through antibody testing in order to better understand the

* Corresponding author: Pontiano Kaleebu, Uganda Virus Research Institute, Plot 51–59, Nakiwogo Road, PO Box 49, Entebbe, Uganda.
E-mail address: pontiano.kaleebu@mrcuganda.org (P. Kaleebu).

https://doi.org/10.1016/j.ijid.2021.09.020

epidemiology of the virus and thereby to introduce effective control measures. Antibody testing has traditionally been conducted using enzyme-linked immunosorbent assays (ELISAs) or more recently with rapid diagnostic tests (RDTs). ELISAs facilitate the testing of large numbers of specimens per run, while RDTs are lateral flow devices for individual specimens. RDTs typically give results in less than 30 minutes and are therefore ideal for use at the point of care (POC). During the early days of the pandemic, commercial ELISAs and RDTs were hard to come by and none were approved for use in Uganda.

Most people infected with SARS-CoV-2 have an incubation period of 3–7 days before the appearance of symptoms. IgM seroconversion occurs within 10–14 days and IgG seroconversion within 12–14 days after symptom onset (Long et al., 2020; Lou et al., 2020; To et al., 2020; Zhao et al., 2020) and can be detected in less than 40% of infected people within 1 week of symptom onset and in 100% by day 15 (Batra et al., 2020; Zhao et al., 2020). Antibodies can take much longer to develop in those with a subclinical or mild infection (WHO, 2020b). The strength of the antibody response depends on a number of factors including age, nutritional status, and disease severity amongst others (WHO, 2020a). IgM antibodies start to disappear by week 5, and by week 7 they are no longer detectable; IgG antibodies persist beyond week 7 (Xiao et al., 2020). It is not clear whether antibodies confer immunity to re-infection, although the recurrence of COVID-19 illness appears to be very uncommon (CDC, 2020a).

As a result of global shortages of reagents for molecular testing, a number of groups (Lassaunière et al., 2020; Zhao et al., 2020) have investigated the potential use of antibody tests, particularly those for IgM, either singly or in combination, to diagnose acute SARS-CoV-2 infection. However, a reliable diagnosis of infection by antibody testing is only possible in the recovery phase when the possibility of intervening has passed and consequently serological diagnosis is not recommended for informing clinical management or contact-tracing (WHO, 2020b).

In Uganda, as in many other countries, there is a sense of urgency to understand the epidemiology of the virus in order to implement effective control measures. This requires mass screening of the population for anti-SARS-CoV-2 antibodies to determine among other things: how many people have been infected with the virus and how this changes over time; the risk factors for infection such as age, ethnicity, domicile, or underlying health issues; the proportion of infected people with mild or asymptomatic infection and how long antibodies can be detected in individuals who have been infected (CDC, 2021). Many countries are now testing for SARS-CoV-2 antibodies at the population level or in specific groups, such as health workers, close contacts of known cases, or within households (WHO, 2020c).

The tools to conduct mass serological screening, including ELISA and RDT kits with emergency use approval, slowly became available in mid-2020 and plans were made to evaluate their performance.

Laboratory tests including RDTs that detect antibodies to SARS-CoV-2 in people need validation to determine their accuracy and reliability. Inaccurate RDT results would have serious consequences and would affect pandemic control efforts. In Uganda, all new diagnostic assays that are introduced into the market must undergo in-country laboratory validation at the Uganda Virus Research Institute (UVRI), which is a designated WHO and Africa Centres for Disease Control and Prevention (CDC) SARS-CoV-2 reference laboratory, before being recommended to the Ministry of Health for use in the country. Both the US Centers for Disease Control and Prevention, and WHO also advise that diagnostic and antibody tests should be validated in appropriate populations and settings before they are recommended (CDC, 2020b; WHO, 2017).

For this evaluation, ELISAs and chemiluminescent microparticle immunoassays (CMIA) with one or more approvals under the US Food and Drug Administration (FDA), Emergency Use Authorization (EUA) or the WHO Emergency Use Listing (EUL), or with the European Conformité Européenne (CE) mark were procured (supplementary files, Appendix A). For the rapid tests, local distributors provided 25 serological RDTs for a cost-free evaluation at the UVRI (supplementary files, Appendix B). Nine distributors provided enough test kits for the evaluation of 150 samples, while 16 distributors provided test kits for less than 150 samples (73–125).

2. Materials and methods

2.1. Serological reference panel

At UVRI, the normal practice to validate new antibody test kits is to evaluate their performance on a reference panel of well-characterized plasma specimens. In mid-2020, no commercial or WHO reference panels were available and consequently UVRI developed its own reference panel. Presumptive anti-SARS-CoV-2 antibody-positive specimens were selected from available qRT-PCR-confirmed, symptomatic and asymptomatic SARS-CoV-2 cases; the number of days post symptom onset was reported for some of the symptomatic cases. Presumptive SARS-CoV-2 antibody-negative specimens were selected from the UVRI repository of specimens collected during an HIV national serosurvey conducted in 2016, long before the COVID-19 pandemic.

Specimens from qRT-PCR-confirmed SARS-CoV-2-positive cases were tested using the following six assays in accordance with the manufacturers' 'information for use' (IFU) instructions: (1) Architect SARS-CoV-2 IgG CMIA (nucleocapsid protein); (2) Architect SARS-CoV-2 IgM CMIA (spike protein); (3) Euroimmun Anti-SARS-CoV-2 ELISA (IgG) (spike protein); (4) EDI Novel Coronavirus COVID-19 IgG ELISA (nucleocapsid protein); (5) EDI Novel Coronavirus COVID-19 IgM ELISA (nucleocapsid protein); (6) InBios SCoV-2 Detect IgM ELISA (spike protein). A positive sample was defined as a sample reactive on at least two IgG ELISA/CMIA targeting the spike protein (Euroimmun Anti-SARS CoV-2 ELISA-IgG) and the nucleocapsid protein (Architect SARS-CoV-2 IgG CMIA) and also reactive on at least two IgM ELISA/CMIA targeting the spike protein (InBios SCoV-2 Detect IgM ELISA and Architect SARS-CoV-2 IgM CMIA). The EDI Novel Coronavirus COVID-19 IgG and IgM ELISA had low sensitivity and hence results with these assays were not considered during the selection of specimens for the positive panel.

Fifty samples with the above profile were included in the SARS-CoV-2-positive reference panel (UVRI, 2021).

SARS-CoV-2-(presumed) negative specimens were tested using the following four assays in accordance with the manufacturers' IFUs: (1) InBios-SCoV-2 Detect IgM ELISA (spike protein); (2) Euroimmun Anti-SARS CoV-2 ELISA-IgG (spike protein); (3) Architect SARS CoV2-IgG CMIA (nucleocapsid protein); (4) SD Biosensor Standard E COVID-19 Total Ab ELISA (spike protein). A negative sample was defined as a sample that was non-reactive in at least three of the four assays.

One hundred samples with the above profile were included in the SARS-CoV-2-negative reference panel (UVRI, 2021).

2.2. Rapid diagnostic tests

A total of 25 serological RDTs (Appendix B) were evaluated against the characterized serological reference panel. The antigen(s), spike and/or nucleocapsid protein, targeted by the RDTs was not disclosed in the IFUs for most of the RDTs. The majority of RDTs had a single reading window with a control line, an IgM test line, and an IgG test line, while one (Biocredit) had two separate cassettes. Two RDTs had a reading window for IgM/IgG (Sino Care

and Wondfo), while one had reading windows for IgG and IgA/IgM and not IgM (Antai). The manufacturers' IFUs were followed and the results were read by two technicians blinded to each other's results.

2.3. Statistical analysis

The statistician conducting the data analysis was blinded to the RDT identity. Only concordant results between technicians were used to evaluate the performance of the RDTs; inter-reader variability was also documented.

2.3.1. Sensitivity

The sensitivity was calculated as the number of specimens determined as positive by the two technicians for each RDT under evaluation, divided by the number of specimens tested from the positive panel; this was expressed as a percentage.

2.3.2. Specificity

The specificity was calculated as the number of specimens determined as negative by the two technicians for each RDT under evaluation, divided by the number of specimens tested from the negative panel; this was expressed as a percentage.

2.3.3. Accuracy

The accuracy was calculated as the proportion of RDT test results that agreed with the panel source (positive and negative panels) and was expressed as a percentage. The sensitivity, specificity, and accuracy calculations were performed using the proportion command in STATA 15 and confidence intervals (CI) were produced with the Wilson score method (Newcombe, 1998).

Sensitivity, specificity, and accuracy were also determined for combined IgM/IgG (either or both IgM and IgG).

2.3.4. Inter-reader agreement

The observed proportion and level of agreement between the two technicians were examined using the Cohen's kappa statistic. This was generated for each isotype (IgM and IgG) of each RDT evaluated. The level of agreement was categorized as follows: no agreement (<0), slight agreement (0.0–0.20), fair agreement (0.21–0.40), moderate agreement (0.41–0.60), substantial agreement (0.61–0.80), and almost perfect agreement (0.81–1.00) (McHugh, 2012).

2.4. Ethical considerations

The evaluation protocol was reviewed and approved by the Research Ethics Committee of the UVRI and the Uganda National Council for Science and Technology (UNCST). The panels were unlinked to personal identifiers and the results could not be traced to individual patients. Consent to participate and to store samples for future use was also sought.

3. Results

3.1. Performance

Results by individual RDT performance are summarized (in alphabetical order) in Table 1. Most RDTs showed poor performance, with none showing both anti-SARS-CoV-2 IgM and IgG antibody sensitivity and specificity ≥98%. Many RDTs that performed well in at least one reading window showed good reactivity to anti-SARS-CoV-2 IgG antibodies, with 10 having a sensitivity ≥98%, while 13 had a specificity ≥98% (Table 1). Only three RDTs had an anti-SARS-CoV-2 IgM antibody sensitivity ≥98%, while 10 RDTs had a specificity ≥98% for anti-SARS-CoV-2 IgM antibodies. Three RDTs (Autobio, MultiG, and Standard Q) had a sensitivity and a specificity ≥98% to combined IgM/IgG. There were seven RDTs that had an accuracy ≥98% for anti-SARS-CoV-2 IgG antibodies, with three (Boson, Standard Q, and VivaDiag) having an accuracy of 100%. There were four RDTs (Boson, MultiG, Standard Q, and VivaDiag) where both anti-SARS-CoV-2 IgG antibody sensitivities and specificities were ≥98%.

3.2. Inter-reader agreement

There was almost perfect agreement between the two technicians for the determination of IgG in 20/23 RDTs (87.0%) (where there was a reading window for IgG). Four of the RDTs had perfect agreement for IgG, with a kappa statistic of 100% (Biocredit, BTNX, MultiG, Standard Q, and VivaDiag). Agreement in determining IgM was much lower, with only 11/23 tests (47.8%) where there was almost perfect agreement (Table 2).

4. Discussion

The WHO continues to review the evidence on antibody responses to SARS-CoV-2 infection and has published guidance on adjusting public health and social measures for the next phase of the COVID-19 response (WHO, 2019). The development of accurate RDTs for the diagnosis of anti-SARS-CoV-2 IgM and IgG antibodies will benefit epidemiological and surveillance studies in identifying past COVID-19 symptomatic and asymptomatic infections including those in 'hot-spots'. This will serve as an aid in determining the extent of herd immunity, although for how long immunity will last, especially with the appearance of SARS-CoV-2 variants, is not yet known (Aschwanden, 2021). Accurate RDTs detecting the relevant antibodies will benefit vaccine studies in identifying SARS-CoV-2 vaccine responders and for how long one remains immune to the virus.

This evaluation of 25 RDTs showed significant variation in performance, emphasizing the need for more input in research and development in order to come up with more accurate tests. There were only four RDTs that had a sensitivity and specificity ≥98% for anti-SARS-CoV-2 IgG, i.e., Boson, MultiG, Standard Q, and VivaDiag. Of these four RDTs, only Boson had a sensitivity ≥98% for anti-SARS-CoV-2 IgM (sensitivity was 100% and corresponding specificity was 87.0% for anti-SARS-CoV-2 IgM; Table 1). When combining IgM and IgG, this evaluation identified three RDTs (Autobio, MultiG, and Standard Q) with a sensitivity and specificity ≥98% for both IgG and/or IgM.

There was better agreement between the two technicians for result determination of IgG compared to result determination of IgM, with 87.0% having almost perfect agreement for IgG compared to only 47.8% for IgM. Five of the RDTs had perfect agreement for IgG, with a kappa statistic of 100% (Biocredit, BTNX, MultiG, Standard Q, VivaDiag), while there was no such finding for IgM.

Combination IgG/IgM RDTs can provide unclear results given the po tential for cross-reactivity of antibodies with other coronaviruses and the often poor specificity of IgM serological assays (IDSA, 2020). The use of some of these combination IgG/IgM RDTs may not be simple to interpret in the field if only the IgG test component of the RDT gives accurate results. Some RDTs had one cassette with separate IgM and IgG bands, while others had separate cassettes for IgG and IgM. If one used an RDT with a cassette having both IgM and IgG and with IgM having poor specificity, then the final result describing past exposure may be confusing and inaccurate, unless the reader disregards the IgM results altogether.

For the above reasons, it may be more practical to recommend the use of RDTs with both a sensitivity and specificity of ≥98% for IgM and IgG combined; Autobio, MultiG, and Standard Q fit this profile.

Table 1
Field performance (sensitivity, specificity, and accuracy) of 25 rapid diagnostic tests (RDTs) evaluated at UVRI.

RDT	Number evaluated	Isotype	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)
Abbott Panbio	150	IgM	66.2 (57.6–73.8)	90.3 (82.2–94.9)	66.2 (57.6–73.8)
		IgG	98.5 (94.3–99.6)	97.7 (91.0–99.4)	98.5 (94.3–99.6)
		IgM/IgG	92.0 (86.9–95.8)	88.7 (6.3–19.5)	92.0 (86.9–95.8)
Absoludy	100	IgM	16.3 (8.2–30.0)	100 (92.9–100)	58.6 (48.5–68.0)
		IgG	95.9 (84.4–99.0)	98.0 (86.3–99.7)	97.0 (90.9–99.0)
		IgM/IgG	96.0 (84.7–99.0)	98.0 (86.3–99.7)	97.0 (90.9–99.0)
Antai	73	IgG	10 (2.2–35.5)	94.6 (79.7–98.7)	50.7 (39.1–62.2)
		IgG/IgM	16.7 (3.3–54.3)	94.6 (79.7–98.7)	50.7 (39.1–62.2)
		IgG/IgA/IgM	37.9 (21.6–57.6)	79.5 (64.5–89.3)	63.0 (51.2–73.5)
Autobio	100	IgM	37.8 (23.3–55.0)	100 (92.7–100)	73.6 (63.1–81.9)
		IgG	98 (86.3–99.7)	97.8 (85.2–99.7)	97.9 (91.9–99.5)
		IgM/IgG	100 (92.9–100)	98.0 (86.3–99.7)	99.0 (93.0–99.9)
Biocredit	100	IgM	4.2 (1.0–15.9)	98 (86.3–99.7)	52.0 (42.0–61.9)
		IgG	2.0 (0.3–13.7)	100 (92.9–100)	51.0 (41.1–60.8)
		IgM/IgG	4.0 (1.0–15.3)	98.0 (86.3–99.7)	51.0 (41.1–60.8)
BioSpeedia	150	IgM	100 (92.9–100)	10.5 (5.5–19.1)	43.4 (35.2–51.9)
		IgG	100 (92.9–100)	81.9 (72.6–88.6)	88.2 (81.7–92.6)
		IgM/IgG	100 (92.9–100)	20.0 (13.2–29.2)	46.7 (38.7–54.8)
BTNX	125	IgM	89.8 (77.1–95.8)	97.3 (89.7–99.4)	94.4 (88.5–97.3)
		IgG	96.0 (84.7–99.0)	100 (95.1–100)	98.4 (93.7–99.6)
		IgM/IgG	100 (92.9–100)	97.3 (89.7–99.4)	98.4 (93.7–99.6)
Boson	100	IgM	100 (92.7–100)	87.0 (73.2–94.2)	93.7 (86.5–97.2)
		IgG	100 (93.9–100)	100 (92.6–100)	100 (96.2–100)
		IgM/IgG	100 (92.9–100)	87.8 (74.2–94.6)	93.9 (87.0–97.3)
Cellex–q	150	IgM	73.8 (57.9–85.2)	88.2 (79.7–93.4)	83.7 (76.4–89.1)
		IgG	96.0 (84.7–99.0)	98.0 (92.0–99.5)	97.3 (92.9–99.0)
		IgM/IgG	96.0 (84.7–99.0)	87.9 (79.7–93.1)	90.6 (84.7–94.4)
CoronaCHEK	148	IgM	81.1 (64.4–91.0)	100 (96.2–100)	94.7 (89.3–97.5)
		IgG	60.5 (44.7–74.3)	100 (96.2–100)	87.9 (81.4–92.4)
		IgM/IgG	83.0 (68.9–91.5)	100 (96.2–100)	94.5 (89.3–97.2)
Egens	100	IgM	59.6 (44.6–73.0)	93.3 (80.6–97.9)	76.1 (66.1–83.8)
		IgG	68.8 (53.9–80.6)	98.0 (86.0–99.7)	83.5 (74.6–89.7)
		IgM/IgG	69.4 (54.7–81.0)	94.0 (82.4–98.1)	81.8 (72.8–88.3)
Hightop	119	IgM	19.5 (9.8–35.2)	98.6 (90.0–99.8)	69.1 (59.7–77.1)
		IgG	76.6 (61.9–86.8)	98.6 (90.0–99.8)	89.7 (82.5–94.1)
		IgM/IgG	72.0 (57.6–83.0)	97.1 (88.8–99.3)	86.8 (79.0–91.7)
MultiG	100	IgM	22.4 (12.6–36.7)	98.0 (86.3–99.7)	61.6 (51.5–70.8)
		IgG	100 (92.9–100)	98.0 (86.3–99.7)	99.0 (93.0–99.9)
		IgM/IgG	100 (92.9–100)	98.0 (86.3–99.7)	99.0 (93.0–99.9)
Novita	150	IgM	17.4 (8.7–31.7)	100 (96.3–100)	74.0 (66.2–80.5)
		IgG	40 (25.6–56.4)	100 (96.3–100)	82.9 (75.9–88.3)
		IgM/IgG	40 (27.1–54.5)	100 (96.3–100)	80.0 (72.7–85.7)
Orient Gene	150	IgM	80.9 (66.5–90.0)	95.3 (89.2–98.5)	90.9 (84.9–94.7)
		IgG	98.0 (86.3–99.7)	95.9 (89.5–98.5)	96.6 (92.1–98.6)
		IgM/IgG	98.0 (86.3–99.7)	93.9 (87.0–97.3)	95.3 (90.4–97.8)
Really Take	100	IgM	93.5 (80.9–98.0)	100 (92.9–100)	93 (85.9–96.7)
		IgG	97.9 (85.5–99.7)	96 (84.7–99.0)	94 (87.1–97.3)
		IgM/IgG	93.5 (80.9–98.0)	100 (92.9–100)	96.9 (90.6–99.0)
Shanghai Liangrun	119	IgM	0 (0–7.3)	100 (94.7–100)	58.5 (49.3–67.1)
		IgG	93.8 (81.7–98.1)	94.1 (85.0–97.8)	94.0 (87.8–97.1)
		IgM/IgG	91.8 (79.6–97.0)	94.2 (85.2–97.9)	93.2 (86.9–96.6)
Sino Care	100	IgM	NA*	NA	NA
		IgG	NA	NA	NA
		IgM/IgG	47.9 (33.8–62.3)	92 (80.0–97.1)	69 (59.1–77.4)
Standard Q	100	IgM	95.9 (84.4–99.0)	98.0 (86.3–99.7)	97.0 (90.9–99.0)
		IgG	100 (92.9–100	100 (92.9–100)	100 (96.3–100)
		IgM/IgG	100 (92.9–100)	98.0 (86.3–99.7)	99.0 (93.0–99.9)
Tigsun	150	IgM	36.2 (23.4–51.2)	77.5 (67.5–85.1)	63.2 (54.7–71.0)
		IgG	80.9 (66.5–90.0)	90.3 (82.2–94.9)	87.1 (80.4–91.8)
		IgM/IgG	79.6 (65.5–88.9)	73.5 (63.7–81.4)	75.5 (67.8–81.9)
Vazyme	100	IgM	14.3 (6.8–27.6)	81.3 (67.1–90.2)	47.4 (37.5–57.5)
		IgG	100 (92.9–100)	95.9 (84.4–99.0)	98.0 (92.1–99.5)
		IgM/IgG	100 (92.9–100)	80.0 (66.1–89.1)	90.0 (82.2–94.6)
VivaDiag	150	IgM	93.8 (81.7–98.1)	97.0 (90.9–99.0)	95.9 (91.1–98.2)
		IgG	100 (92.9–100)	100 (96.3–100)	100 (97.5–100)
		IgM/IgG	100 (92.9–100)	97.0 (90.9–99.0)	98.0 (93.9–99.4)
Wiz Biotech	150	IgM	98.0 (86.0–99.7)	0 (0–3.7)	32.2 (25.1–40.2)
		IgG	98.0 (86.0–99.7)	0 (0–3.7)	32.2 (25.1–40.2)
		IgM/IgG	98.0 (86.0–99.7)	0 (0–3.7)	32.2 (25.1–40.2)
Wondfo	100	IgM	NA[a]	NA	NA
		IgG	NA	NA	NA
		IgM/IgG	95.8 (84.1–99.0)	98 (86.3–99.7)	96.9 (90.8–99.0)

(*continued on next page*)

Table 1 (*continued*)

RDT	Number evaluated	Isotype	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)
Zybio	100	IgM	93.6 (81.3–98.0)	79.5 (64.5–89.3)	86.8 (78.0–92.4)
		IgG	95.9 (84.4–99.0)	100 (92.6–100)	97.9 (91.9–99.5)
		IgM/IgG	96.0 (84.7–99.0)	81.6 (67.7–90.4)	88.9 (80.9–93.8)

CI, confidence interval; UVRI, Uganda Virus Research Institute.

[a] NA indicates that the sensitivity and specificity of these tests could not be generated separately because the kits did not have separate reading windows for IgM and IgG.

Table 2
The observed proportion of agreement and Cohen's kappa statistic for the 25 rapid diagnostic tests (RDTs)

RDT	Number of kits received for evaluation	Isotype	Observed proportion of agreement (%)	Kappa
Abbott	150	IgM	88.7	0.55
Panbio		IgG	91.3	0.82
Absoludy	100	IgM	99.0	0.94
		IgG	99.0	0.98
Antai	73	IgG	78.1	0.25
		IgG/IgM	67.1	0.15
Autobio	100	IgM	87.0	0.61
		IgG	96.0	0.92
Biocredit	100	IgM	98.0	0.74
		IgG	100	1.0
BioSpeedia	150	IgM	90.7	0.52
		IgG	96.0	0.92
BTNX	125	IgM	99.2	0.98
		IgG	100	1.0
Boson	100	IgM	95.0	0.90
		IgG	98.0	0.96
Cellex-q	150	IgM	90.0	0.77
		IgG	98.7	0.97
CoronaCHEK	48	IgM	89.9	0.73
		IgG	95.3	0.85
Egens	100	IgM	92.0	0.82
		IgG	97.0	0.93
Hightop	119	IgM	92.4	0.63
		IgG	97.5	0.94
MultiG	100	IgM	99.0	0.95
		IgG	100	1.0
Novita	150	IgM	97.3	0.79
		IgG	93.3	0.72
Orient Gene	150	IgM	95.3	0.89
		IgG	98.7	0.97
Really Take	100	IgM	96.0	0.92
		IgG	97.0	0.94
Shanghai Liangrun	119	IgM	99.2	0.0
		IgG	97.5	0.95
Sino Care	100	IgM	NA	
		IgG	NA	
		IgM/IgG	98.0	0.95
Standard Q	100	IgM	99.0	0.98
		IgG	100	1.0
Tigsun	150	IgM	90.7	0.78
		IgG	94.0	0.87
Vazyme	100	IgM	97.0	0.90
		IgG	99.0	0.98
VivaDiag	150	IgM	98.0	0.95
		IgG	100	1.0
Wiz Biotech	150	IgM	99.3	0.66
		IgG	99.3	0.66
Wondfo	100	IgM/IgG	98.0	0.96
Zybio	100	IgM	91.0	0.82
		IgG	97.0	0.94

All of the test kits recommended above for IgG (Boston, Multi G, Standard Q, and VivaDiag) had almost perfect agreement; similarly for either IgG or IgM (IgG/IgM), MultiG and Standard Q had almost perfect agreement, while Autobio had substantial agreement.

To evaluate the performance of the 25 RDTs, a well-characterized reference panel of plasma specimens from SARS-CoV-2 qRT-PCR-positive individuals that had been screened by means of six SARS-CoV-2 ELISA/CMIA IgM and IgG assays was used. This approach is different from many other studies that have used any samples from qRT-PCR-positive individuals. The reason for this approach is that not all qRT-PCR-positive individuals have antibodies; the presence of antibodies depends on the time since infection, the severity of infection, and a number of other factors. A further step to increase the relevance of the reference panel was to include ELISA/CMIA kits that targeted either the nucleocapsid or the spike antigen where possible.

There are other published evaluation reports that have similarly shown poor serological RDT performance compared to that re-

ported by the manufacturer (Deeks et al., 2020; Jacobs et al., 2020; Vauloup-Fellous et al., 2021). Since some of these other studies used samples from any qRT-PCR-positive individuals, they showed lower sensitivity in the first week post symptom onset, with improved performance at later time points. Few studies have evaluated RDTs using samples taken beyond 1 month post symptom onset. Furthermore, there is limited information on the performance of these RDTs in asymptomatic participants (Deeks et al., 2020).

While a serial or orthogonal (Xu et al., 2020) testing approach has been recommended for surveillance especially when using ELISAs (CDC, 2020b), here we propose that parallel testing with RDTs could also be a viable approach. With this approach, two RDTs with ≥98% sensitivity and specificity could be used together with an equally accurate RDT as a tie-breaker for discrepant results.

This study evaluated only 25 RDTs, but we are aware that many SARS-CoV-2 RDT kits are on market. With the well characterized panel of samples we now have and with WHO serological reference standards now available from the National Institute for Biological Standards and Control (NIBSC), the validation of additional RDTs will be quicker.

The study had some limitations. The selection of the RDTs to evaluate was dictated by what was provided to us by the local distributors in the country. Some distributors provided test kits for evaluation, less than the desired 150 samples. Another limitation was that it was not possible to procure an IgM ELISA based on the nucleocapsid protein. Some of the ELISA kits procured did not perform well and hence the results with these kits were excluded from the development of the serological reference panel. Subsequent to the completion of this evaluation, it was discovered that authorization for the use of some of the RDTs had been revoked; they were removed from the FDA EUA notification list as of February 23, 2021.

In conclusion, using a reference panel of well characterized plasma samples and considering the sensitivity and specificity of combined IgG and IgM results, and inter-reader agreement, this evaluation found that three RDTs performed well: Autobio, MultiG, and Standard Q. Where only the IgG result is of interest, there were four RDTs that performed well: Boson, MultiG, Standard Q, and VivaDiag.

Contributors

Tom Lutalo TL, Jennifer Serwanga JS, Aminah Nalumansi AM, Bernard Kikaire BK, Dennis Olara DO, Deogratius Ssemwanga DS, John Kayiwa JKa, Brendah Abiko BA, Bernard Ogwang BO, Christopher Nsereko CN, Emmanuel Odwilo EO, Matthew Cotten MC, Christine Watera CW, Joshua Buule JB, Stephen Balinandi SB, Julius Lutwama JL, Jocelyn Kiconco JKi, Robert Downing RD, Joweria Nakaseegu JN, Pontiano Kaleebu PK.

Author contributions

TL, RD, PK, AN, JS, BK, DS, MC, JK, and PK conceived and designed the study. TL, RD, and PK wrote the first draft. TL, AN, DO, JKa, BO, EO, CW, SB, JKi, JN, JS, BK, DS, BA, CN, MC, JB, JL, RD, and PK provided specimens and/or demographic data and/or conducted tests and/or interpreted the data. JKa, JKi, JN, JL, and JB conducted the molecular testing. AN, DO, BO, EO, and BA conducted the antibody testing. TL and BO handled the data and analysis. All authors reviewed/revised the manuscript and gave final approval for submission.

Acknowledgements

We thank the individuals who provided the specimens, including those at the different COVID-19 treatment centers. We acknowledge Uganda Government funding to the UVRI. The procurement of the SARS-CoV2 qRT-PCR kits was funded mainly through the WHO and Africa CDC by different donors, Jack Ma Foundation, and the UK Medical Research Council (MRC)/UK Research and Innovation. Part of the funding from the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) is under the MRC/DFID Concordat Agreement and is also part of the EDCTP2 programme supported by the European Union.

Funding: No specific funding was received from funding bodies in the commercial, public, or not-for-profit sectors.

Conflict of interest: All authors declare no competing interests.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2021.09.020.

References

Aschwanden C (2021) Five reasons why COVID herd immunity is probably impossible. https://www.nature.com/articles/d41586-021-00728-2.

Batra R, et al. A comparative evaluation between the Abbott Panbio™ COVID-19 IgG/IgM rapid test device and Abbott Architect™ SARS CoV-2 IgG assay. Journal of Clinical Virology 2020;132. doi:10.1016/j.jcv.2020.104645.

CDC (2020a) CDC's Diagnostic Test for COVID-19 Only and Supplies. https://www.cdc.gov/coronavirus/2019-ncov/lab/virus-requests.html.

CDC (2020b) Interim guidelines for COVID-19 antibody testing in Clinical and Public Health Settings. https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html.

CDC (2021) COVID-19 Serology Surveillance Strategy. https://www.cdc.gov/coronavirus/2019-ncov/covid-data/serology-surveillance/.

Deeks JJ et al. (2020) Antibody tests for identification of current and past infection with SARS-CoV-2 Cochrane Database of Systematic Reviews doi:10.1002/14651858.CD013652

IDSA (2020) IDSA COVID-19 Antibody Testing Primer. https://www.idsociety.org/globalassets/idsa/public-health/covid-19/idsa-covid-19-antibody-testing-primer.pdf.

Jacobs J, Kühne V, Lunguya O, Affolabi D, Hardy L, Vandenberg O (2020) Implementing COVID-19 (SARS-CoV-2) Rapid Diagnostic Tests in Sub-Saharan Africa: A Review Frontiers in Medicine 7 doi:10.3389/fmed.2020.557797

Lassaunière R, Frische A, Harboe ZB, Nielsen ACY, Fomsgaard A, Krogfelt KA, Jørgensen CS (2020) Evaluation of nine commercial SARS-CoV-2 immunoassays medRxiv:2020.2004.2009.20056325 doi:10.1101/2020.04.09.20056325

Long Q-X, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nature Medicine 2020;26:845–8. doi:10.1038/s41591-020-0897-1.

Lou B et al. (2020) Serology characteristics of SARS-CoV-2 infection since the exposure and post symptoms onset medRxiv:2020.2003.2023.20041707 doi:10.1101/2020.03.23.20041707

McHugh ML. Interrater reliability: the kappa statistic. Biochem Med (Zagreb) 2012;22:276–82.

Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. Statistics in Medicine 1998;17:857–72.

To KK-W, Tsang OT-Y, Leung W-S, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. The Lancet 2020;20:565–74. doi:10.1016/S1473-3099(20)30196-1.

UVRI (2021) COVID-19 Panel Raw Data. https://www.uvri.go.ug/sites/default/files/project-reports/UVRI_COVID-19_Pos-Neg-Panel_For_Kit_Evaluation_May2021.pdf.

Vauloup-Fellous C, et al. Performance of 30 commercial SARS-CoV-2 serology assays in testing symptomatic COVID-19 patients. European journal of clinical microbiology & infectious diseases: official publication of the European Society of Clinical Microbiology 2021:1–7. doi:10.1007/s10096-021-04232-3.

WHO (2017) WHO Bulletin:A guide to aid the selection of diagnostic tests. https://www.who.int/bulletin/volumes/95/9/16-187468.pdf.

WHO (2019) Considerations in adjusting public health and social measures in the context of COVID-19. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/critical-preparedness-readiness-and-response-actions-for-covid-19

WHO (2020a) Advice on the use of point-of-care immunodiagnostic tests for COVID-19. https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19.

WHO (2020b) Diagnostic testing for SARS-CoV-2: interim guidance. https://apps.who.int/iris/handle/10665/334254.

WHO (2020c) Unity Studies: Early Investigation Protocols

WHO (2021) WHO Coronavirus (COVID-19) Dashboard. https://covid19.who.int/?adgroupsurvey={adgroupsurvey}&gclid=Cj0KCQjwjPaCBhDkARIsAISZN7SnRhAcjZNWDTLfiW4eu0ojxSqLb0sMZtmbsns6Z8RmOf-dAEquD3MaAtkNEALw_wcB.

Xiao AT, Gao C, Zhang S. Profile of specific antibodies to SARS-CoV-2: The first report. Journal of Infection 2020;81:147–78. doi:10.1016/j.jinf.2020.03.012.

Xu G, et al. Evaluation of Orthogonal Testing Algorithm for Detection of SARS-CoV-2 IgG. Antibodies Clinical Chemistry 2020;66:1531–7. doi:10.1093/clinchem/hvaa210.

Zhao J, et al. Antibody Responses to SARS-CoV-2 in Patients With Novel Coronavirus Disease 2019. Clinical Infectious Diseases 2020;71:2027–34. doi:10.1093/cid/ciaa344.