Evaluation of a rapid test against two ELISAs for a SARS-CoV-2 seroprevalence survey in Kibera informal settlement, Nairobi, Kenya

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Abstract

Introduction The performance of a rapid test was evaluated against two ELISAs as a potentially useful tool to determine exposure to SARS-CoV-2 in low resource settings. A serosurvey was conducted in Kibera informal settlement, Nairobi, Kenya, where low numbers of COVID-19 were recorded during the pandemic. **Materials and methods** A cross-sectional study was performed in 10 of 14 villages in Kibera informal settlement, Kenya's largest slum community, in August 2021, before general vaccine roll-out. Participants were age one year and above with no symptoms of COVID-19. Capillary blood samples were tested using the Standard Q COVID-19 IgM/IgG Combo rapid test, Platelia SARS-CoV-2 Total Ab (IgM/IgG/IgA) Assay, and Wantai Total Ab (IgM/IgG/IgA) ELISA for SARS-CoV-2 which served as the reference test. **Results** Samples were obtained from 438 participants; in 72 samples blood was insufficient for the Platelia ELISA. Specificity of the rapid test and Platelia ELISA were similar (>93%) but sensitivity was low (rapid test 61.3%; Platelia ELISA 83.4%). The Wantai ELISA showed greater positivity (82.6%) than the rapid test (51.8%) and Platelia ELISA (69.7%). **Conclusions** The Wantai ELISA showed superior performance in this serosurvey. Point-of-care tests for convenient screening for SARS-CoV-2 exposure for surveillance studies need to be developed.

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Conclusions

The Wantai ELISA showed superior performance in this serosurvey. Point-of-care tests for convenient screening for SARS-CoV-2 exposure for surveillance studies need to be developed.

Key words: serosurvey, COVID-19, SARS-CoV-2 antibody, rapid diagnostic test, ELISA

Introduction

The World Health Organization declared severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of coronavirus disease 2019 (COVID-19,) a pandemic in March 2020. In Kenya, the first confirmed case was reported in March 2020 [1]; COVID-19 vaccination was initiated in March 2021. By July 2023, 343,898 confirmed cases and 5,689 deaths were recorded since the pandemic start [2], rates that remained low compared with global figures. Kibera informal settlement in Nairobi, one of sub-Saharan Africa's largest slums, where COVID-19 prevention measures were impossible to implement, reported especially low disease rates. This study evaluated the performance of a rapid test as a potentially useful serosurveillance tool to determine previous SARS-CoV-2 infection in a low resource setting.

Materials and Methods

Subjects

Consented participants age one year and above with no current symptoms suggestive of COVID-19 were tested; COVID-19 vaccination status was collected.

Materials

Three antibody tests were performed: Standard Q COVID-19 IgM/IgG Combo rapid test (SD Biosensor Inc, Suwon, Gyeonggi, Republic of Korea), Platelia SARS-CoV-2 Total Ab (IgM/IgG/IgA) Assay (Bio-Rad Laboratories Inc, California, USA), and Wantai Total Ab (IgM/IgG/IgA) ELISA for SARS-CoV-2 (Wantai Biological Pharmacy Enterprise Co. Ltd, Beijing, China).

Methods

500 µL capillary blood was collected by fingerprick from each participant into an EDTA-coated microtainer tube. Blood samples were transported to the Kenya Medical Research Institute laboratories for same-day testing using the rapid test; blood was separated and plasma samples stored at –80 °C for ELISA testing. The rapid test has three lines on the nitrocellulose membrane: "C" control line, and "G" and "M" test lines. Monoclonal anti-chicken IgY antibody is coated onto the control line region, and monoclonal anti-human IgG antibody and monoclonal anti-human IgM antibody onto the "G" and "M" test line regions. Recombinant COVID-19 nucleocapsid protein conjugated with colloidal gold particles are used as detectors for the "M" and "G" test lines. SARS-CoV-2 antibodies in the specimen combine with recombinant COVID-19 nucleocapsid protein conjugated with colloidal gold particles, producing an antibody–antigen gold particle complex which migrates to the "M" and "G" test lines, and is captured by monoclonal anti-human IgG or IgM antibodies. A violet test line appears in the results window if SARS-CoV-2 antibodies are present in the specimen. 20 µL capillary blood was applied to the specimen well of the test device using the capillary tube provided. Three drops (90 µL) of buffer were added to the specimen well and the test result read at 10–15 minutes. For valid tests, a coloured band was observed at the C test line; the test was considered positive if coloured bands appeared at the M test line (IgM), G test line (IgG), or both.

The Wantai SARS-CoV-2 Ab ELISA is a two-step incubation antigen "sandwich" enzyme immunoassay for qualitative detection of antibodies to the SARS-CoV-2 spike protein receptor binding domain. Polystyrene

microwell strips are pre-coated with recombinant SARS-CoV-2 antigen; during the first incubation, SARS-CoV-2 antibodies present in the sample are captured in the wells. The microwells are washed to remove unbound serum proteins, and recombinant SARS-CoV-2 antigen conjugated to horseradish peroxidase enzyme (HRP conjugate) added; conjugated antigen binds to captured antibody inside the wells during a second incubation. The microwells are washed to remove unbound conjugate, and chromogen solution added; in wells containing the antigen–antibody–antigen "sandwich" immunocomplex, colourless chromogens are hydrolysed by bound HRP conjugate to a blue coloured product which turns yellow after stopping the reaction with sulphuric acid. 100 µL of plasma sample was added to each well of the microwell plate and incubated at 37 °C for 30 minutes. Each well was washed to remove unbound antibody before adding 100 µL HRP-conjugated recombinant SARS-CoV-2 antigen, and re-incubated. Following a second cycle of washing, 50 µL each of chromogen solution A and B were added to each well and further incubated. The colour intensity of positive samples was measured using a wavelength of 450 nm with the cut-off at 1 nm.

The Platelia SARS-CoV-2 Total Ab Assay is a one-step antigen capture format assay using wells pre-coated with recombinant SARS nucleocapsid protein. Samples are pre-diluted and mixed with recombinant SARS nucleocapsid protein coupled with peroxidase (conjugate), and incubated in the wells. IgM, IgG and/or IgA antibodies present in the specimen form a complex between recombinant SARS-nucleocapsid protein and recombinant SARS-nucleocapsid protein coupled with peroxidase. After washing, the presence of immune complex is demonstrated after adding a chromogenic solution for colour development. 15 µL of plasma from each sample was pre-diluted and added to each well with conjugate, and incubated for one hour at 37° C. After a washing step, the colour development solution was added and incubated for 30 minutes at room temperature. The optical density (OD) was read at 450 nm within 30 minutes of adding stopping solution, with the cut-off calculated at 1 nm.

Statistical analysis

Data were entered into Excel and analysed using SAS version 9.4 (SAS Institute Inc). The results of the three tests were compared using cross-tabulations. ELISA indeterminate results (OD 0.9-0.99 nm) were considered "not positive". Sensitivity (positive result in a true positive case (TP)), specificity (negative result in a true negative case (TN)), positive and negative predictive values (PPV, NPV) (the probability of positive or negative results in true positive or negative cases, respectively) and positive and negative likelihood ratios (LRP and LRN) were calculated for the rapid test and Platelia ELISA using the Wantai ELISA as the reference test, which has shown superior performance in previous evaluations against RT-PCR confirmed samples [3,4]. Standard formulae were used: sensitivity (TP)/(TP + FN) (FN = false negative); specificity (TN)/(TN + FP) (FP = false positive; positive predictive value (TP/TP + FP); negative predictive value (TN/TN + FN); and positive and negative likelihood ratios (LR+, LR-) (probability that a person with or without the disease tested positive respectively; and probability that a person with or without the disease tested negative respectively). Results were calculated within 95% confidence intervals. The Kappa coefficient of agreement between the rapid test and the Wantai ELISA, and between the Platelia ELISA and the Wantai ELISA, were interpreted according to the criteria of Cohen (values [?]0 indicating no agreement; 0.01–0.20 as none to slight agreement; 0.21–0.40 as fair agreement; 0.41–0.60 as moderate agreement; 0.61–0.80 as substantial agreement; 0.81–1.00 as almost perfect agreement) [5]. The results of the total population were compared with those of the non-vaccinated population.

Results

The serosurvey was conducted from 2–13 August 2021. Capillary blood samples were obtained from 438 participants; 72 samples was insufficient to conduct the Platelia ELISA, the last test conducted. Table 1 shows the comparisons between the tests. Table 2 shows the sensitivity, specificity, PPV and NPV, LR+ and LR-, and kappa coefficient of agreement of the rapid test and Platelia ELISA against the Wantai ELISA. The Wantai ELISA showed greater percentage positive results (82.6%) compared with the rapid test (51.8%) and the Platelia ELISA (69.7%). Of the rapid test results, 23 were IgM positive, 151 were IgG positive; 53 were both IgM and IgG positive. Only 30 (6.8%) participants reported one or more COVID-19 vaccine doses up to five months before the study, with no difference in percentage positive results or kappa values of

agreement between the total and non-vaccinated population.

Discussion

This study evaluated the performance of one rapid test and two ELISAs for detecting antibodies to SARS-CoV-2 in asymptomatic individuals in a slum community in Nairobi, Kenya, using capillary blood samples. Most evaluations of serological tests have been carried out by comparison with RT-PCR in both positive and negative COVID-19 cases. In a systematic review by Lisboa Bastos of serological test performance in 40 studies, pooled sensitivity of rapid tests was 66.0% (95% CI 49.3%-79.3%), and of ELISAs measuring IgG or IgM was 84.3% (95% CI 75.6%-90.9%) [6]. In all analyses, pooled sensitivity was lower for rapid tests, the potential point-of-care method; pooled specificity range was 96.6%-99.7%. Sensitivity of commercial rapid tests (49.0%-78.2%) was lower than of non-commercial tests (83.6%-91.3%). Sensitivity was higher at least three weeks after symptom onset (69.9%–98.9%) than in the first week (13.4%–50.3%). Ghaffari in another systematic review including both rapid tests and ELISAs reported greater variability in sensitivity than in specificity, and suggested serological tests were more effective in the later stages of disease [7].

Typically in response to viral infection, IgM is produced first, with a later switch to IgG production for long-term immune memory [8]. However, one study showed no statistical difference for IgM or IgG seropositivity between testing samples taken from PCR-confirmed COVID-19 cases between 9–17 days and 18–29 days [9]; studies of SARS-associated coronaviruses suggest IgM and IgG often develop around the same time [10,11]. It may therefore not be possible to judge recency of infection in this population group. Studies have also shown IgM and IgG levels are significantly higher in severe COVID-19 cases than in patients with mild or moderate disease [12], suggesting serological tests require high sensitivity to detect antibodies in mild or asymptomatic cases. A Cochrane meta-analysis of antibody studies concluded there was no certainty about how well the tests would work in asymptomatic or milder disease cases [13].

The rapid test showed fair (0.32) and Platelia ELISA moderate (0.6) agreement with the Wantai ELISA. As with other studies, specificity of the rapid test was high (93.42%) but sensitivity lower (61.33%); the Platelia ELISA similarly showed good specificity (93.85%) but poorer sensitivity (83.39%). The number of vaccinated individuals was too small to influence the results. More accurate point-of-care tests for field-based screening for SARS-CoV-2 exposure in population surveys are needed.

Ethics approval and consent to participate

Ethics approval was obtained from Amref Health Africa Ethics and Scientific Review Committee, the National Commission for Science Technology and Innovation, and Nairobi Metropolitan Services, Ministry of Health, Kenya. Verbal permission to conduct the study was obtained from five Kibera area chiefs, the local government administration covering the 10 villages. Informed consent was obtained from all participants, parents or caregivers, and assent from children age 12–17 years.

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Author Contributions

Conceptualisation: JYC, SK, SM; training: JYC, SMM, SM, SK; Field work SM, SK, JK, NL, JC, AM, RO; writing—original draft preparation: JYC, SK, SM; writing, review and editing: JYC, SK, SM, SMM, LK, RS, MH, MP, JS, JO. All authors have read and agreed to the published version of the manuscript.

Competing interests

All authors declare they have no competing interests.

Data sharing

After de-identification, all individual participant data and underlying results reported in this article are available for anyone who wishes to access the data for any purpose.

REFERENCES

1. Aluga, MA. Coronavirus disease 2019 (COVID-19) in Kenya: Preparedness, response and transmissibility. J Microbiol, Immun, and Infect. 2020; 53(5), 671. https://doi.org/10.1016/J.JMII.2020.04.011
2. World Health Organization. Kenya - WHO Coronavirus (COVID-19) Dashboard. https://covid19.who.int > region > afro > country
3. Hanssen DAT, Slaats M, Mulder M, Savelkoul PHM, van Loo IHM. Evaluation of 18 commercial serological assays for the detection of antibodies against SARS-CoV-2 in paired serum samples. Eur J Clin Microbiol Infect Dis. 2021;40:1695–703.
4. Harritshoj LH, Gybel-Brask M, Afzal S, Kamstrup PR, Jorgensen CS, Thomsen MK, *et al.*Comparison of 16 serological SARS-CoV-2 immunoassays in 16 clinical laboratories. J Clin Microbiol. 2021;59:e02596–20.
5. Cohen, J. (1960). A Coefficient of Agreement for Nominal Scales. Educational and Psychological Measurement, 20(1), 37–46. https://doi.org/10.1177/001316446002000104
6. Lisboa Bastos M, Tavaziva G, Abidi SK, Campbell JR, Haraoui L-P, Johnston JC, *et al.*Diagnostic accuracy of serological tests for COVID-19: Systematic review and meta-analysis. BMJ. 2020;370:m2516.
7. Ghaffari A, Meurant R, Ardakani A. COVID-19 serological tests: How well do they actually perform? Diagnostics (Basel). 2020 Jul 4;10(7):453. doi: 10.3390/diagnostics10070453. PMID: 32635444; PMCID: PMC7400479.
8. Azkur AK, Akdis M, Azkur D, Sokolowska M, van de Veen W, Bruggen MC, *et al.* Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy. 2020 Jul;75(7):1564-1581. doi: 10.1111/all.14364. PMID: 32396996; PMCID: PMC7272948.
9. Hoffman T, Nissen K, Krambrich J, Ronnberg B, Akaberi D, Esmaeilzadeh M, Salaneck E, Lindahl J, Lundkvist A. Evaluation of a COVID-19 IgM and IgG rapid test; an efficient tool for assessment of past exposure to SARS-CoV-2. Infect Ecol Epidemiol. 2020 Apr 14;10(1):1754538. doi: 10.1080/20008686.2020.1754538. PMID: 32363011; PMCID: PMC7178815.
10. Li G, Chen X, Xu A. Profile of specific antibodies to the SARS-associated coronavirus. N Engl J Med. 2003 Jul 31;349(5):508-9. doi: 10.1056/NEJM200307313490520. PMID: 12890855.
11. Hsueh PR, Huang LM, Chen PJ, Kao CL, Yang PC. Chronological evolution of IgM, IgA, IgG and neutralisation antibodies after infection with SARS-associated coronavirus. Clin Microbiol Infect. 2004 Dec;10(12):1062-6. doi: 10.1111/j.1469-0691.2004.01009.x. PMID: 15606632; PMCID: PMC7129952.
12. Yu HQ, Sun BQ, Fang ZF, Zhao JC, Liu XY, Li YM, *et al.* Distinct features of SARS-CoV-2-specific IgA response in COVID-19 patients. Eur Respir J. 2020 Aug 27;56(2):2001526. doi: 10.1183/13993003.01526-2020. PMID: 32398307; PMCID: PMC7236821.
13. Fox T, Geppert J, Dinnes J, Scandrett K, Bigio J, Sulis G, *et al.* Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Database of Systematic Reviews 2022, Issue 11. Art. No.: CD013652. DOI: 10.1002/14651858.CD013652.pub2. Accessed 24 August 2023.

Table 1. Cross-tabulation of Standard Q COVID-19 IgM/IgG Combo rapid test, Platelia SARS-CoV-2 Total Ab (IgM/IgG/IgA) Assay, and Wantai Total Ab (IgM/IgG/IgA) ELISA test results for SARS-CoV-2 in a seroprevalence study (Kibera Informal Settlement; Nairobi, Kenya). The Wantai ELISA is defined as the reference test.

	Wantai SARS-CoV-2 Ab ELISA	Wantai SARS-CoV-2 Ab ELI
	Total n=438	Total n=438
Standard Q COVID-19 IgM/IgG Combo rapid test	Positive	Negative
Positive	222	5
Negative	140	71
Total	362	76
Platelia SARS-CoV-2 Total Ab Assay	n=366	n=366
Positive	251	4
Negative	50	61

	Wantai SARS-CoV-2 Ab ELISA	Wantai SARS-CoV-2 Ab ELI
Total	301	65
	Platelia SARS-CoV-2 Total Ab Assay	Platelia SARS-CoV-2 Total A
	n=366	n=366
Standard Q COVID-19 IgM/IgG Combo rapid test	Positive	Negative
Positive	173	27
Negative	82	84
Total	255	111

Table 2. Performance of rapid test and Platelia ELISA against the reference test (Wantai ELISA)

	Total	**Total**	**Unvaccinated**	**Unvaccinated**
	Rapid test (95% CI)	**Platelia ELISA (95% CI)**	**Rapid test (95% CI)**	**Platelia ELISA (95**
Sensitivity (%)	61.33 (56.31–66.34)	83.39 (79.18–87.59)	59.46 (54.19–64.73)	83.70 (79.34–88.05)
Specificity (%)	93.42 (87.85–98.99)	93.85 (88.00–99.69)	93.33 (87.69–98.98)	93.75 (87.32–99.68)
PPV (%)	97.80 (95.89–99.71)	98.43 (96.91–99.96)	97.54 (95.40–99.67)	98.30 (96.64–99.95)
NPV (%)	33.65 (27.27–40.02)	54.95 (45.70–64.21)	34.15 (27.66–40.64)	57.14 (47.68–66.61)
LR+	9.32 (3.98–21.83)	13.55 (5.24–35.06)	8.92 (3.81–20.90)	13.39 (5.18–34.64)
LR–	0.41 (0.36–0.48)	0.18 (0.14–0.23)	0.43 (0.38–0.50)	0.17 (0.13–0.23)
Kappa	0.32 (0.25–0.39)	0.60 (0.51–0.70)	0.32 (0.24–0.39)	0.62 (0.53–0.71)

CI=confidence intervals; PPV=positive predictive value; NPV=negative predictive value; LR+=positive likelihood ratio; LR-=negative likelihood ratio