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Age significantly influences the sensitivity of SARS-CoV-2 rapid antibody assays

Natalie Irwin¹,², Lyle Murray¹,², Benjamin Ozynski²,³, Guy A Richards¹,², Graham Paget¹,², Jacqueline Venturas¹,², Ismail Kalla¹,², Nina Diana¹,², Adam Mahomed¹,², Jarrod Zamparini¹,²,*

¹ Department of Internal Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
² Department of Medicine, Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg, South Africa
³ Wits Health Innovation, Johannesburg, South Africa

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BACKGROUND: Point-of-care serological assays are a promising tool in COVID-19 diagnostics but do have limitations. Our study evaluated the sensitivity of five rapid antibody assays and explored factors influencing their sensitivity in detecting SARS-CoV-2-specific IgG and IgM antibodies.

METHODS: Finger-prick blood samples from 102 participants, within 2–6 weeks of PCR-confirmed COVID-19 diagnosis, were tested for IgG and IgM using five rapid serological assays. The assay sensitivities were compared, and patient factors evaluated in order to investigate potential associations with assay sensitivity.

RESULTS: Sensitivity ranged from 36% to 69% for IgG and 13% to 67% for IgM. Age was the only factor significantly influencing the likelihood of a detectable IgG or IgM response. Individuals aged 40 years and older had an increased likelihood of a detectable IgG or IgM antibody response by rapid antibody assay.

CONCLUSION: Rapid serological assays demonstrate significant variability when used in a real-world clinical context. There may be limitations in their use for COVID-19 diagnosis among the young.

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BACKGROUND

Rapid serological assays, used at the point of care (POC), pose a promising clinical tool in the diagnosis of coronavirus disease 2019 (COVID-19), particularly in low- and middle-income countries where diagnostic resources are scarce. These lateral flow chromatographic immunoasays qualitatively detect immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on a venous or finger-prick whole-blood sample without the need for specialized equipment. Such assays are useful for rapid antibody testing in surveillance programmes in outbreak settings or in high-epidemiological areas. The assays may assist in the diagnosis of suspected COVID-19 in patients who test negative for SARS-CoV-2 by polymerase chain reaction (PCR) on naso- or oropharyngeal swabs (Theel, 2020). In addition, they require minimal operator training and have a turnaround time of under 30 minutes (Ricció, 2020).

Some valid concerns about the performance quality of these rapid assays exist, with most available rapid assays having been subjected only to single-centre internal validation studies, using small populations (Department of Health, 2020). Furthermore, the threshold antibody titer required to generate a detectable result on these devices is poorly described. Reported overall IgG/IgM sensitivities range from 18.4% to 93.3%, and vary according to disease severity and duration since symptom onset (Ricció, 2020).

Our study critically evaluated the sensitivity of five rapid antibody assays for the detection of SARS-CoV-2-specific IgM and IgG antibodies, using finger-prick blood samples from patients with COVID-19 confirmed by PCR on nasopharyngeal or oropharyngeal swab. Importantly, this study also investigated patient factors that influenced the sensitivity of these assays.

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* Correspondence to: Dr Jarrod Zamparini, Area 552, Division of Infectious Diseases, Department of Medicine, Charlotte Maxeke Johannesburg Academic Hospital, Parktown, Johannesburg 2193, South Africa.
E-mail address: jarrod.zamparini@wits.ac.za (J. Zamparini).
METHODS AND MATERIALS

This study was approved by the University of the Witwatersrand Human Research Ethics Committee (Medical) (M200697). Written informed consent was obtained from all participants, and patient data were anonymized prior to analysis.

Study participants

Adult participants (≥18 years old) were recruited at the Charlotte Maxeke Johannesburg Academic Hospital in Johannesburg, South Africa. Randomly selected inpatients and outpatients were invited to participate if they had laboratory-confirmed COVID-19 by RT-PCR on a naso- or oropharyngeal swab prior to interview and testing. Participant numbers were limited by the number of assay cassettes available.

Clinical and biographical data were collected using an electronic database (REDCap 10.6.2, Vanderbilt University) by means of a self-administered participant questionnaire. Variables collected included demographics (age, sex, self-reported ethnicity), comorbidities, and whether the participant was a healthcare worker (HCW). Participants provided details of previous PCR testing, including the number of previous tests done, the date and result of each test, the route of sampling (oropharyngeal or nasopharyngeal), symptoms experienced at the time of the positive test, the date of onset of symptoms, and the severity of disease (asymptomatic, mild, moderate, severe, critical) classified according to published criteria (National Institutes of Health, 2020)

Rapid antibody assays

Five rapid immunochromatographic antibody assays were evaluated in this study and performed for each participant:

1. 2019-nCoV-IgG/IgM Rapid Test (whole blood, serum, or plasma), Lot 200505, Dynamiker Biotechnology Company Ltd, Tianjin, China (Dynamiker)
2. 2019-nCoV IgG/IgM Rapid Test Cassette (whole blood, serum, or plasma), Lot NPC20030123, AllTest Biotech Company Ltd, Hangzhou, China (AllTest)
3. 2019-nCoV Ab Test (Colloidal Gold) (serum, plasma, or venous whole blood), Lot 20200402, Innovita Biotechnology Company Ltd, Tanshang, China (Innovita)
4. Medical Diagnostech COVID-19 IgG/IgM Rapid Test (whole blood, serum, or plasma), Lot 200703, Altis Biologics (Pty) Ltd, Pretoria, South Africa (Altis)
5. Cellex qSARS-CoV-2 IgG/IgM Cassette Rapid Test (serum, plasma, or whole blood), Lot W11006-DH-GZ-20200511, Cellex, Jiangsu, China (Cellex)

A single drop (10–20 μL) of whole blood drawn by fingertip puncture was deposited in the sample well of each test cassette. Two to five drops of reagent buffer were then added to the sample well and results read 15–20 minutes later according to the specific manufacturer's instructions. Assays 3 and 5 had not previously been validated on finger-prick specimens. Two readers (N.I. and/or J.Z. and/or B.O.) read the cassettes with the naked eye, while a third reader (J.V.) settled any disputes. A test was considered valid if a control line was visualized and was interpreted as positive if the control line and the line for IgG or IgM, or both, were seen. Each of the five kits was evaluated once on each participant, with no replication of testing on any participants. All test cassettes and reagents for each of the five different kits were from the same manufacturing batch.

Table 1

| Participant demographics | Total cohort, n (%) | Clinical severity, n (%) |
|--------------------------|---------------------|-------------------------|
| HCW                      | 69 (68%)            | Asymptomatic 8 (8%)     |
| Age (yrs)*               | 37.5 (29–45.75)     | Mild 61 (60%)           |
| Gender, n (%)            |                     | Moderate 20 (20%)       |
| Male                     | 40 (39%)            | Severe/critical 13 (13%)|
| Female                   | 62 (61%)            | Location of treatment   |
| Race, n (%)              |                     | Inpatient 18 (18%)      |
| Black                    | 41 (40%)            | Outpatient 84 (82%)     |
| White                    | 35 (34%)            | Comorbidities, n (%)    |
| Indian                   | 22 (22%)            | Hypertension 14 (14%)   |
| Mixed race               | 4 (4%)              | Asthma 10 (10%)         |
|                          |                     | Diabetes 12 (12%)       |
|                          |                     | HIV 6 (6%)              |
|                          |                     | Cancer 3 (3%)           |

HCW = healthcare worker; *median (IQR)
Clinical severity based on the NIH Guidelines (National Institutes of Health, 2020).

Statistical analysis

Data were analyzed using Prism 8.4.3 (GraphPad Software Inc., La Jolla, California) using standard non-parametric statistical tests, as appropriate. Continuous data were expressed as medians with interquartile ranges (IQRs) and categorical variables presented as numbers and percentages. Fisher’s exact tests were used to compare results in the age, time, and severity groups, and Spearman’s correlation coefficients were used to assess agreement between test assays. The multivariate logistic regression analysis was performed using IBM SPSS Statistics 26.0 (IBM Corporation, Armonk, New York).

RESULTS

Demographics

Antibody testing was conducted using all five rapid antibody assays on 102 participants with previous PCR-confirmed COVID-19. The majority of our participants were female (61%) and of black ethnicity (40%). Median age in the cohort was 37.5 years (IQR 29–45.75) and most of the participants were HCWs (68%). The majority of our cohort were tested between 15 and 42 days after positive PCR testing (n = 83, 81%), with four (4%) and 15 (15%) being tested less than 15 days and more than 42 days post-PCR-testing, respectively. Comorbidities among the participants included hypertension (n = 14; 14%), diabetes (n = 12; 12%), asthma (n = 10; 10%), HIV (n = 6; 6%), and cancer (n = 3; 3%). A small number of the patients in our cohort required inpatient management for COVID-19 (n = 18, 18%), in keeping with the number of participants classified as having severe disease. Additional demographic data are presented in Table 1.

Antibody assay sensitivity

Overall sensitivity in detecting SARS-CoV-2-specific IgG and IgM antibodies was below 70% for all assays (Figure 1). IgG sensitivity ranged from 36% (Innovita) to 69% (Dynamiker), while IgM sensitivity ranged from 13% (Innovita) to 67% (Dynamiker). Of note, the sensitivities of the Innovita (13%), AllTest (15%), and Altis (26%) assays in detecting IgM were markedly lower than those of the Dynamiker (67%) and Cellex (64%) assays.

Variability between assays

Variability in diagnostic accuracy between the five rapid antibody assays for the detection of IgG and/or IgM was assessed through the use of a correlation matrix represented in a heat map
was in agreement with a study by Wu et al., which showed significantly higher neutralizing antibody titers in elderly and middle-aged patients when compared with young patients (Wu F, 2020).

Gender

Male gender has previously been associated with COVID-19 severity (Peckham, 2020). However, we did not find any differences in sensitivity based on gender within our cohort for any of the rapid antibody assays for IgG or IgM (Tables 2 and 3).

Time since positive SARS-CoV-2 PCR test

Our hypothesis was that the dynamic antibody responses during and after COVID-19 may influence the detection of IgG and IgM SARS-CoV-2-specific antibodies (Zhao, 2020). The association between time since SARS-CoV-2 PCR test and the detection of IgG and IgM antibodies was explored. There was no difference in IgG sensitivity between those presenting within 30 days of a positive PCR test compared with those presenting after 30 days, as shown in Table 2. However, a significantly lower proportion of individuals tested more than 30 days after a positive SARS-CoV-2 PCR test had a detectable IgM response with the AllTest assay (23% ≤ 30 days) vs 6% (> 30 days), p = 0.02, OR = 0.2228 (0.0644–0.7993); Table 3). There was no significant difference in IgM sensitivity between the two time groups for the other four rapid antibody assays (p > 0.05 for Dynamiker, InnovaT, Alitis, and Cellex; Table 3).

In order to better define the temporal relationship between time elapsed since COVID-19 and the sensitivity of the rapid assays in detecting SARS-CoV-2-specific IgG and IgM antibodies, individuals were further stratified according to time since positive SARS-CoV-2 PCR test, and the differences in sensitivities of the assays between these strata assessed (Figure 3). There was great heterogeneity in sensitivity for detecting IgM among the assays, with no consistent pattern of sensitivity according to time since positive PCR test. Contrasting patterns of sensitivity for IgM according to time were shown by the Dynamiker and AllTest assays. There was a significantly higher sensitivity for detection of IgM in individuals testing more than 40 days after a positive PCR test when compared with those testing after less than 21 days when using the Dynamiker assay (88.9% (> 40 days) vs 64% (< 21 days), p = 0.046) (Figure 3). By contrast, when using the AllTest assay, the sensitivity for IgM was lower in the group testing more than 40 days after a positive PCR test (0% (> 40 days) vs 24% (< 21 days), p = 0.032)

(Figure 2). This measured agreement between each assay for the detection of IgG or IgM. Four of the five assays (Dynamiker, AllTest, Alitis, and Cellex; r > 0.8 for all; Figure 2) correlated well with each other in the detection of IgG. However, none of these four assays correlated well with the InnovaT assay for IgG. Strong correlation for IgM results was found between only two of the five assays (Dynamiker and Cellex; r > 0.8; Figure 2). The correlation results were all statistically significant at p < 0.05.

Analysis of the factors associated with improved sensitivity of the rapid antibody assays

Participant factors potentially associated with the sensitivity of detection of IgG or IgM by rapid antibody assay were investigated. These included: gender, time since positive SARS-CoV-2 PCR test (≤ 30 days vs > 30 days), age (< 40 years vs ≥ 40 years) and COVID-19 disease severity (asymptomatic-mild vs moderate-severe).

Age

Most strikingly, there was a significant reduction in IgG sensitivity for participants under 40 years of age compared with those over 40 years for all of the assays used (Table 2; p < 0.005 for all). Similarly, there was a significant reduction in IgM sensitivity in those under 40 years of age compared with those over 40 years of age; however, this significance was only demonstrated in four of the five assays (Table 3; p < 0.005 for all except the AllTest).
considered all; was protecting influence Disease (21–30 days), no significant difference in sensitivity between the two groups (p > 0.05 for all; Table 3).

**Disease severity**

COVID-19 disease severity has been shown to impact the magnitude of the SARS-CoV-2-specific antibody response (Zhao, 2020). Our study therefore investigated whether disease severity had an influence on the sensitivities of the rapid antibody assays in detecting IgG or IgM antibodies to SARS-CoV-2. Interestingly, there was no significant difference in IgG sensitivity between those with asymptomatic or mild disease and those with moderate or severe disease for each assay (Table 2). There was also no significant difference in IgM sensitivity between the two groups (p > 0.05 for all; Table 3).

**Multivariate analysis**

A multivariate logistic regression was performed to exclude confounding variables and to confirm the factors associated with an increased likelihood of a detectable SARS-CoV-2-specific IgG or IgM response by rapid antibody assay. The variables included age, gender, time since positive test, and disease severity. Age > 40 years was the only variable associated with a significantly increased likelihood of a detectable IgG and IgM response by rapid antibody testing (Supplementary Tables 1 and 2). This was significant for all of the rapid antibody assays for IgG and all except the AllTest assay for IgM (Supplementary Tables 1 and 2).

**DISCUSSION**

Rapid serological assays are increasingly becoming an essential component of the surveillance of outbreaks and retrospective diagnoses of COVID-19. These assays have appeal because they are rapid, inexpensive, and user-friendly. However, data evaluating their true clinical performance on finger-prick analyses at the POC, in a real-world clinical context, are urgently needed.

![Figure 2. Heat map showing correlations between rapid antibody assays for the detection of SARS-CoV-2-specific IgG and IgM. The Spearman r coefficient, representing the degree of agreement between each pair of rapid antibody assays, is shown in each square for IgG (left panel) and IgM (right panel). A Spearman r value greater than 0.8 is considered a significant agreement. All values shown had significant p-values of < 0.05.](image)
In this study, when tested against the reference gold standard PCR, the sensitivities of five rapid antibody assays ranged from 36% to 69% for IgG and from 13% to 67% for IgM. This was significantly lower than those reported in previous validation studies of these rapid tests (Dynamiker Biotechnology (Tianjin) Co., Ltd., 2020; Mølbak, 2020; Lassaunière, 2020). The South African Health Products Regulatory Authority (SAHPRA) specifies that a minimum clinical sensitivity of 85%, within 95% confidence intervals, be proven prior to registration (SAHPRA, 2020). All of these rapid antibody assays fell below that benchmark when used in our cohort. The wide spread of results for our sensitivity analysis was comparable with the findings of previous studies. In a pooled analysis of 1030 POC antibody assays by Riccò et al., combined sensitivity for IgG and IgM ranged from 18.4% to 93.3%, with an average of 64.8% (95% CI 54.5–74.0) (Riccò, 2020). Our study also reported poor correlation among the results of the five assays, particularly when testing for IgM. This finding has also been previously described, and prompted Van Elslande et al. to question the need for measuring IgM SARS-CoV-2 antibodies at all (Cassaniti, 2020; Van Elslande, 2020). The heterogeneous clinical sensitivity performance of assays to IgM antibodies in our study further suggest limited clinical utility. Given the poor correlation between the Innovita assay and all other kits, we would not recommend use of this kit on finger-prick blood samples.

Our analysis of patient factors associated with improved sensitivity of the rapid antibody tests revealed a link between age and sensitivity. All of the assays demonstrated improved sensitivities in those patients aged > 40 years for IgG. For IgM the same was true, except for the Altest assay. The higher sensitivities of these tests in older participants may indicate higher antibody titers in these individuals, and therefore a higher likelihood of antibody detection. Older patients with COVID-19 have been shown to have higher SARS-CoV-2-specific IgG and IgM antibody titers than younger patients, although the reason for this is unclear (Huang, 2020; Wu F, 2020). It is likely that increased SARS-CoV-2-specific antibody titers correlate with more severe COVID-19, and that increased age is correlated with more severe disease and worse outcome (Zhao, 2020; Zhou, 2020). When both age and disease severity were included in a multivariate logistic regression analysis, only age was associated with an increased likelihood of a positive rapid IgG or IgM antibody response, thereby suggesting that age may play a role independently of its association with disease severity. Our cohort consisted primarily of participants who had mild COVID-19, with 80% of the cohort in the mild-to-moderate category. Because younger individuals typically experience milder COVID-19 disease and have an increased rate of asymptomatic infection, our findings may suggest a significant impediment to the use of these assays in assessing seroprevalence in younger participants.

The impact of time since diagnosis of COVID-19 by positive PCR test on the sensitivity of the rapid assays was intriguing. An association between increased sensitivity in detecting IgG and in-
creased since positive PCR was as expected, and was consistent with previous studies (Deeks, 2020; Wu JL, 2020). For IgM, the two kits with the highest sensitivity in detecting IgM (Dynamiker and Celllex) showed most sensitivity when tested among individuals more than 40 days after the PCR test. Although generally believed to be relatively short-lived, SARS-CoV-2-specific IgM antibodies have been shown to persist for up to two-and-a-half months after SARS-CoV-2 infection, which may explain this finding (Iyer, 2020). It is not clear whether poor IgM sensitivity in some of the assays is associated with higher specificity; further studies, possibly with the use of ELISA assays, would be required to investigate this.

There are limitations to this study. Although used as the reference gold standard for the diagnosis of COVID-19, PCR-based testing gives no indication of patient seropositivity. The negative serological results reported here may represent low antibody titers or low participant seroconversion, rather than a failure of the test to detect antibodies. Furthermore, two of the included assays (Innovita and Celllex) had not previously been validated with finger-prick blood samples. Although we consider that the effect is likely to be small, as the other tests could be used across all blood sample platforms, this may have contributed to their poor performance. Owing to inadequate access to negative control samples, we were not able to perform our own corresponding specificity analysis. However, a Cochrane Diagnostic Test Accuracy Review has shown such rapid serological assays to have very high specificity (Deeks, 2020).

CONCLUSION

This study described an overall underperformance of rapid serological assays in detecting an IgG and IgM response 2–6 weeks after PCR-confirmed SARS-CoV-2 infection. The results highlighted the heterogeneous ability of the antibody assays to detect IgM, and described a significant independent association between age > 40 years and increased sensitivity for IgG and IgM seropositivity. Judicious clinical use, recognizing the limitations of rapid serological assays, especially among the young, is necessary.

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Conflicts of interest

The authors declare no conflicts of interest.

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Meeting(s) where the data have been presented

These data have not been previously presented at any meetings or conferences.

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