Submission of research report for graduation for the degree

MMed in Chemical Pathology

03/02/2023

TITLE:

VALIDATION OF ROCHE IMMUNOASSAY FOR SEVERE ACUTE RESPIRATORY VIRUS 2/ SARS-COV-2 IN SOUTH AFRICA

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DECLARATION

I, Jurette Simone Grove, student nr 445823, a candidate for the degree of MMed in Chemical Pathology, have submitted my research report entitled:

Validation of Roche immunoassay for severe acute respiratory virus 2/SARS-COV-2 in South Africa.

I declare that:

1. This research report was submitted with the acquiescence of the supervisors.

2. All of the work is my own, except as otherwise stated.

3. The substance (nor any part of it) has not been submitted in the past nor is being submitted for a degree in any other university

4. This research report has the approval of the Committee for Research on Human Subjects, and the Number of the Certificate of Approval is M200694

Signed 

Date 31/01/2023

TABLE OF CONTENTS

1. PUBLISHED ARTICLE	p 1 - 6
2. RESEARCH PROTOCOL	p 7 – 18
3. ETHICS CLEARANCE	p 19
4. TURN-IT IN REPORT	p 20 - 64

Southern African Journal of Infectious Diseases
ISSN: (Online) 2313-1810, (Print) 2312-0053

Validation of Roche immunoassay for severe acute respiratory coronavirus 2 in South Africa

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Background: Serology testing is an important ancillary diagnostic to the reverse transcriptase polymerase chain reaction (RT-PCR) test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We aimed to evaluate the performance of the Roche Elecsys™ chemiluminescent immunoassay (Rotkreuz, Switzerland), that detects antibodies against the SARS-CoV-2 nucleocapsid antigen, at an academic laboratory in South Africa.

Methods: Serum samples were collected from 312 donors with confirmed positive SARS-CoV-2 RT-PCR tests, with approval from a large university's human research ethics committee. Negative controls included samples stored prior to December 2019 and from patients who tested negative for SARS-CoV-2 on RT-PCR and were confirmed negative using multiple serology methods (n = 124). Samples were stored at –80 °C and analysed on a Roche cobas™ 602 autoanalyser.

Results: Compared with RT-PCR, our evaluation revealed a specificity of 100% and overall sensitivity of 65.1%. The sensitivity in individuals > 14 days' post-diagnosis was 72.6%, with the highest sensitivity 31–50 days' post-diagnosis at 88.6%. Results were also compared with in-house serology tests that showed high agreement in majority of categories.

Conclusions: The sensitivity at all-time points post-diagnosis was lower than reported in other studies, but sensitivity in appropriate cohorts approached 90% with a high specificity. The lower sensitivity at earlier time points or in individuals without symptomatology may indicate failure to produce antibodies, which was further supported by the comparison against in-house serology tests.

Keywords: COVID-19; SARS-CoV-2; serology; antibodies; validation; immunoglobulin G; immunoglobulin M.

Introduction

The coronavirus disease 2019 (COVID-19) is a viral infection caused by severe acute respiratory coronavirus 2 (SARS-CoV-2), a novel coronavirus first identified in Wuhan, China in December 2019. It has subsequently caused a global pandemic, infecting more than 126 million people and resulting in the death of more than 2.7 million individuals worldwide.[1,2]

Whilst non-pharmacological methods such as social distancing can limit the spread of the disease, there is a need for rapid identification of infected individuals not only for diagnosis but also to prevent further transmission.[3]

The gold standard for acute SARS-CoV-2 diagnosis is the reverse transcriptase polymerase chain reaction (RT-PCR) performed on an oropharyngeal or nasopharyngeal swab sample. The acceptable turnaround time for this test in South Africa is 24 h – 48 h, but the large burden of disease and worldwide shortage of test kits have constrained availability, resulting in prolonged turnaround times worldwide.[4] Further limitations of RT-PCR include that detection relies on the presence of the viral genome in sufficient amounts for amplification.[5]

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Project research number: R14/49

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Dates: Received: 02 Apr. 2021 | Accepted: 02 June 2021 | Published: 26 July 2021

How to cite this article: Grove JS, Mayne ES, Burgers WA, et al. Validation of Roche immunoassay for severe acute respiratory coronavirus 2 in South Africa. S Afr J Infect Dis. 2021;36(1), a286. https://doi.org/10.4102/sajid.v36i1.286

Missing the window of viral replication and incorrect sampling may produce false-negative results.[5,6] There is therefore a demand for additional testing strategies.

Serological tests that identify antibodies produced in response to infection have the potential for a rapid turnaround time.[7] Although the extent and timing of the humoral response against SARS-CoV-2 is still under investigation, immunoglobulin M (IgM) and A (IgA) antibodies directed at one or more of the major structural proteins (membrane, envelope, spike and nucleocapsid) are generally detectable at a median of day 5 and immunoglobulin G (IgG) antibodies at a median of day 14 post-symptom onset.[5] Immunoglobulin M antibody levels drop after about day 14 when the IgG antibody levels start to rise. There is potential for serological assays to be utilised in a variety of ways: to assist with diagnosis of the disease together with RT-PCR,[8] to identify past infections including in paediatric patients with multisystem inflammatory syndrome of COVID (MIS-C),[9] to perform seroprevalence studies,[4] to assess the immune response to a potential COVID-19 vaccine and, lastly, to identify donors for convalescent plasma.[10]

Since the identification of serology methods as an ancillary diagnostic, there has been a rapid development of a wide range of different assays.[4]

A review of 40 studies indicated that chemiluminescent immunoassays (CLIA) methodology had the highest sensitivity with a pooled sensitivity of 97.8%, compared with enzyme linked immunosorbent assay (ELISA) with 84.3% and lastly lateral flow immunoassays (LFIA) with 66.0%.[11] Roche Diagnostics (Rotkreuz, Switzerland) developed an electrochemiluminescent immunoassay (Elecsys™ Anti-SARS-CoV-2), which detects total antibodies against the SARS-CoV-2 nucleocapsid antigen, although it is most specific for IgG and IgM. Results are reported in a qualitative manner: below the cut-off index (COI) of 1 is interpreted as non-reactive, compared with a reactive result that is equal or greater than the COI of 1.

The manufacturer claims that this assay has a specificity of 99.81%, with a 100% sensitivity after day 14.[12] There have been multiple validations of the assay as set out in Table 1. Importantly, sensitivity is generally reported at time points postinfection of 14 days or more when IgG antibodies are more likely to be produced.

Limitations of many of these evaluations are that numbers of positive participants sampled were small and individuals tested were predominantly symptomatic. Their performance has also not been extensively examined at time points postinfection of 30 days or more raising questions regarding the persistence of an immunological response to the virus.

Of note, there have been limited validations in the African context. International studies suggest that patients of African descent are disproportionately likely to have severe disease and to die.[17] South Africa is currently the epicentre of the African pandemic with 1 545 979 cases and 52 710 deaths (29 March).[18] This prompted the evaluation of ancillary diagnostic methods in the African setting.

The auto-laboratory at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and the National Health Laboratory Service (NHLS) is a large tertiary referral laboratory servicing the Johannesburg area and surroundings. This laboratory operates a Roche cobas 602 (Rotkreuz, Switzerland). Our aim was to validate the Roche Elecsys™ electrochemiluminescent immunoassay and to assess the immune response based on symptomatology and number of days' post-molecular diagnosis and to identify the appropriate use case of this testing in South Africa.

Material and methods

Subjects

This prospective analytical study was conducted at the NHLS based at a large tertiary hospital's laboratory between May and August 2020. Patients who tested positive for SARS-CoV-2 with RT-PCR on a nasopharyngeal swab within South Africa were invited to take part in the study. At the time of this study, there was a global shortage of RT-PCR reagents because of the increased demand for testing. As a result of the aforementioned problem, it was not possible to include RT-PCR results utilising only one uniform reagent. Instead, positive RT-PCR that utilised one of the following reagents were included: Allplex™ 2019 nCoV assay (Seegene, Korea) that targets E, ribonucleic acid-dependant polymerase (RdRP) and N genes; TaqPath™ COVID-19 V2 assay (Applied Biosystems by ThermoFisher Scientific, United States of America) that targets open reading frame of 1ab (ORF1ab), S and N genes; LightMix® Modular SARS and Wuhan CoV E-gene kit (TIB Molbiol for Roche Diagnostics, Switzerland) that targets RdRp and E genes and lastly the Abbott Alinity m SARS-CoV-2 assay (Abbott Molecular, United States) that targets N and RdRP gene. A positive RT-PCR was defined as two or more gene targets identified as positive, with a cycle threshold (Ct) of ≤ 37, as these samples were collected before

TABLE 1: Cumulative review of validations of Roche Elecsys™ severe acute respiratory virus syndrome coronavirus 2 assay.

Location	Number of positive samples included	Total number of positive participants	Number of negative controls	Reported sensitivity > 14 days post-positive RT-PCR (%)	Reported specificity (%)	Reference
Belgium	140	97	79	91.1	100.0	Favresse et al.[13]
Singapore	349	205	715	97.1	99.9	Lau et al.[14]
Germany	186	58	88	89.1	100.0	Hörber et al.[15]
Taiwan	346	74	194	97.4 (> 21 days)	99.0	Chen et al.[16]

RT-PCR, reverse transcriptase polymerase chain reaction.

the National Institute of Communicable Diseases' (NICD) guideline to report a positive result if one or more gene targets were identified as positive. Majority of the positive RT-PCR samples, and specifically all samples where uncertainty existed about the method that was used, had a repeat confirmatory test conducted on the same sample on the Gene Xpert (Cepheid, Sunnyvale, United States) platform within 7 days. Samples were stored for 7 days at –80 °C, and samples that could not be verified were excluded from the study. Venous blood samples were obtained after consent in a serum separator or Ethylenediaminetetraacetic acid (EDTA) tube (BD Vacutainer™) because both serum and plasma were acceptable for the platform. After centrifugation, plasma or serum was extracted, aliquoted and subsequently frozen at –80 °C, with freeze-thaw cycles limited to one. Negative control samples included remnant samples from patients stored prior to December 2019 and from patients who tested negative for SARS-CoV-2 on RT-PCR, confirmed to be negative on other serology methods, particularly in-house anti SARS-CoV-2 ELISAs, to mitigate the risk of using a false negative sample.

Data were also collected on the age and symptomatology of participants, if available and consented.

Methods

For this study we investigated the Roche Diagnostics Elecsys™ Anti-SARS-CoV-2 electrochemiluminescent immunoassay (Rotkreuz, Switzerland) that detects total antibodies against SARS-CoV-2.

Evaluation of the analytical performance was carried out in accordance with the Clinical and Laboratory Standards Institute (CLSI) EP 12 document and the United States' Food and Drug Administration (FDA) guidelines.[19,20]

Initial assay optimisation was performed on 93 patient samples prior to commencing with the full clinical validation. In the absence of standardised quality control (QC) material commercially available at the time, positive controls were derived from three positive pooled patient samples and negative controls from five negative pooled samples, as recommended by the manufacturer.

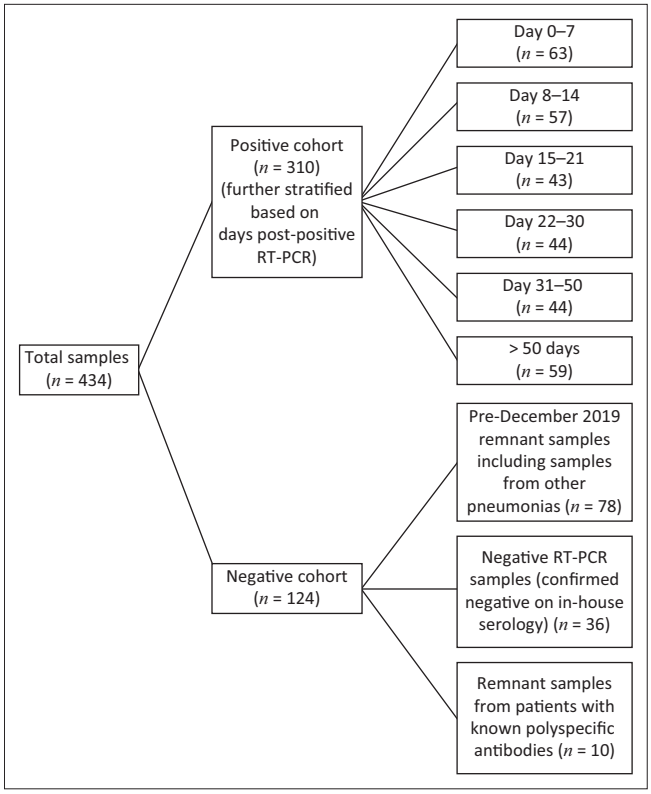
The clinical validation consisted of 434 participants' samples. This included negative samples (n = 124) and positive samples (n = 310). The positive samples were further stratified based on the number of days post RT-PCR diagnosis (Figure 1).

Samples were thawed and run on the Roche cobas™ e602 module. Although the risk of a false positive RT-PCR was mitigated by repeating analysis on the Gene Xpert platform, as discussed here, comparison against RT-PCR as the gold standard is inherently flawed because a positive RT-PCR does not guarantee a positive serology result. To address the potential misclassification of samples as false negatives, that were in fact true negatives in patients with positive RT-PCR that did not produce antibodies, a comparison was performed against a composite serology platform consisting of a Western blot and immunofluorescence, that utilised insect cell-expressed recombinant full length N and S antigens and an in-house serology ELISA assay utilising plant-based recombinant spike 1 (S1) and receptor binding domain (RBD) antigens, in collaboration with another large university.[21] After completion on the Roche platform, all samples were sent for analysis on the composite serology platform. Unfortunately, because of sample volume constraints, only 216 out of the 434 samples had sufficient results on the composite serology platform to form part of the comparison against this method. This consisted of 33 negative samples and 183 positive samples. A result was deemed as positive if 2/3 or 3/3 methods yielded a positive result.

Inter-run precision was carried out in line with the CLSI recommendation, although it had to be modified to run over 3 days instead of 5 because the on-board stability of the reagent at the time of validation was only 72 h.

Statistical analysis

Results were compared with both the RT-PCR and disaggregated by days post-diagnosis. Samples were further stratified by symptom score, which was assigned as follows: 0 for asymptomatic, 1 for mild diseases indicating only respiratory tract symptoms, 2 for moderate disease indicating symptoms outside of the respiratory tract and 3 for severe disease requiring admission. Samples were further compared with in-house serology, to assess the presence of detectable



RT-PCR, reverse transcriptase polymerase chain reaction.

FIGURE 1: Summary of samples used in validation.

TABLE 2: Sensitivity of stratified data – Days post positive reverse transcriptase polymerase chain reaction and symptoms, compared to reverse transcriptase polymerase chain reaction and agreement compared with composite serology.

Stratified data days post positive RT-PCR	Sensitivity compared to RT-PCR (%)	95% confidence interval (%)	Agreement compared with composite serology (%)	95 % confidence interval (%)
0–7 days	52.4	39.4–65.1	84.6	54.6–98.1
8–14 days	54.4	40.7–67.6	77.8	40–97.2
15–21 days	72.1	56.3–84.7	88.2	63.6–98.5
22–30 days	47.7	32.5–63.3	66.7	34.9–90.1
31–50 days	88.6	75.4–96.2	100.0	86.3–100
> 14 days	72.6	65.7–78.8	93.3	85.9–97.5
> 50 days	79.7	75.4–96.2	100.0	86.3–100
Symptom score				
0 (asymptomatic)	57.6	39.2–74.5	81.8	48.2–97.7
1 (mildly symptomatic)	59.2	44.2–73	82.6	61.2–95
2 (moderately symptomatic)	64.4	53.7–74.3	91.7	80–97.7
3 (severely symptomatic)	69.8	57–80.7	90.9	58.7–99.8
Stratified data – Days post-PCR in symptomatic individuals				
> 14 days in moderately to severely symptomatic	81	70.6–89	97.6	87.4–99.9

RT-PCR, reverse transcriptase polymerase chain reaction.

TABLE 3: Sensitivity with the asymptomatic cohort removed.

Stratified data days post positive RT-PCR (days)	Sensitivity compared with RT-PCR without the asymptomatic cohort (%)	95% confidence interval (%)
0–7	47.6	32–63.6
8–14	50	34.2–65.8
15–21	85.7	67.3–95.8
22–30	61.3	42.2–78.2
31–50	90	73.5–97.9
> 14	76.5	67.7–83.9
> 50	70	48.2–85.7

RT-PCR, reverse transcriptase polymerase chain reaction.

antibodies of results because the limitations of RT-PCR are well known (including the ability to detect past infection).

Data analysis

Statistical analysis was performed with MedCalc statistical software version 19.4.1 (MedCalc Software Ltd., Belgium). We defined sensitivity as the proportion of correctly identified COVID-19-positive patients who were positive by RT-PCR SARS-CoV-2 analysis in respiratory samples, whilst specificity was defined as the proportion of correctly identified negative samples. Sensitivity was also reported according to symptom score and days post positive RT-PCR.

Inter-run precision was calculated with Microsoft Excel as CV (%) = (standard deviation [SD] × 100)/mean, and expressed as a percentage coefficient of variation.

Ethical considerations

Ethical approval to conduct the study was obtained from the Human Research Ethics Council of the University of the Witwatersrand (reference number: M200694). Informed consent was obtained from all participants.

Results

The median age of both cohorts was 42, the positive cohort (n = 310) with a range of 19–87 years (minimum to maximum), compared with the negative control (n = 124) of 22–75 years. Both cohorts had a slight female preponderance (61.6% in the positive group and 57.1% in the negative group). By symptom category, moderately symptomatic participants were the largest group (n = 90) and asymptomatic participants were the smallest group (n = 33). Participants were almost equally distributed by number of days post diagnosis, except for those between 0 and 7 days who made up 20% of the total cohort (n = 63).

Accuracy analysis

The specificity of the assay was high at 100% (95% confidence interval [CI] 97.07% – 100%). The overall sensitivity of the Roche assay across all participants was 65.2% (95% CI 59.57% – 70.46%) when compared with RT-PCR results.

Samples were also stratified by days post RT-PCR and degree of symptoms and compared with both the RT-PCR and the in-house composite serology platform (Table 2). Comparison against RT-PCR demonstrated that sensitivity was greatest at > 14 days post PCR in severely symptomatic participants. Comparison against in-house serology demonstrated much higher agreement in all groups, with 100% > 30 days post positive RT-PCR.

Compared with the in-house serology, the overall positive agreement was 89.4% (95% CI 82.18% – 94.39%), with a slight reduction in negative agreement of 88.4% (95% CI 80.53% – 93.83%). The asymptomatic group demonstrated the lowest sensitivity or agreement across both comparisons.

As the asymptomatic group revealed the lowest sensitivity, analysis of the data was repeated with removal of asymptomatic patients from all cohorts, which revealed an increase in the sensitivity in all the groups > 14 days, apart from a slight decline in > 50 days, as set out in Table 3.

Precision analysis

In order to assess inter-run repeatability, 98 of the samples were tested in duplicate, with a qualitative repeatability result

of 100%. Ten samples with results around the COI of one were identified and also tested in duplicate, with 100% agreement.

The QC material, made up as recommended by the manufacturer, was tested five times over 3 days to accommodate the short on-board reagent stability. The assay showed acceptable precision with an index value of 0.093 and a coefficient of variation of 1.5% in the negative controls, and an index value of 3.14 and a coefficient of variation (CV) of 2.2% in the positive controls. This broadly agrees with the % CV of other immunoassays performed on the cobas e602.[14,22]

Samples from patients with known autoimmune disease and poly-specific immunoglobulins, obtained before February 2020, were also analysed (n = 10), with 100% specificity. Seven of these samples were tested in duplicate, with 100% result concordance.

Discussion

This study aimed to evaluate the test performance of the Roche Elecsys Anti-SARS-CoV 2 assay tested on a cobas 602 in a high prevalence setting in South Africa. Results were compared with RT-PCR as the gold standard. This revealed a variable sensitivity ranging from 47.8% to 88.6% and a high specificity of 100%, the latter in line with the manufacturer's claims.[12]

These findings support the use of this assay for the case proposed including for retrospective diagnosis and for seroprevalence studies because a high diagnostic specificity is essential in these settings. There was no cross-reactivity observed with samples that had poly-specific antibodies. Sensitivity was moderately low in individuals more than 14 days' post-positive test result at 72.63%, but improved in patients with moderate or severe symptoms. This test demonstrated the highest sensitivity compared with RT-PCR in severely ill or admitted individuals at time points of 14 days post-diagnosis although this failed to reach a level of 100% sensitivity after 14 days in any group as claimed by the manufacturer.[12] This study found low sensitivity of the assay in asymptomatic patients and in patients before 14 days post-diagnosis, which agrees with findings from other validations internationally. This reflects the dynamics of the humoral response to SARS-CoV-2, which suggests that IgG antibodies are only detectable on day 10 post-infection and reach a maximum after day 14 and IgM antibodies are produced at about day 5–7 but are transient and are not produced by all patients.[5,13,15,23] This is further supported by the high agreement in these time points compared with composite serology. There was a slight decline in sensitivity seen at day 50, but this was insignificant. Although few studies of antibody persistence have been published, there are suggestions in some cases that antibody levels may decline after 2 months and that IgM particularly is undetectable after 30–60 days in most patients.[24,25] The poor performance in day 22–30 post diagnosis could not be fully explained, although only a third of the patients with known symptomatology were classified as severe, which may have skewed the data in this participant group. This was supported by the increase in sensitivity seen after reanalysis without the asymptomatic patients in the group, although an important confounder remained that symptomatology was not known for a fifth of the group. Importantly, sensitivity remained low when compared with the in-house ELISA and the possibility exists that this indicates that anti-N antibodies were not produced in this subset.

Previous studies have shown that antibodies against the N- and S-protein are produced more or less at the same time, however there is a need for further data to evaluate if antibodies against both are produced in all subjects.[23]

This study is the most extensive evaluation of the Roche Elecsys Anti SARS-CoV-2 electrochemiluminescence immunoassay, assessing all time points post-positive RT-PCR, but most specifically more than 21 days post-positive diagnosis and correlating results with symptomatology. The assay had a high specificity in line with global validations and manufacturer claims. This validation showed the lowest sensitivity in the asymptomatic symptom group, which seems to confirm that not all individuals infected with SARS-CoV-2 produce systemic antibodies, especially if asymptomatic.[26] Cumulative sensitivity and sensitivities at all the time points compared with RT-PCR as gold standard, were lower than other comparable studies reported globally, but the inclusion of asymptomatic participants in all groups may offer a partial explanation for this decline.[13,14,15,16] The agreement in all groups significantly improved with comparison against in-house serology. Importantly, the viral and humoral response dynamics indicate that these two assays are sensitive at different time points in infection with the RT-PCR more sensitive prior to day 14 and the serology more sensitive thereafter. This supports the use of these tests for the indications that are approved in South Africa including for retrospective diagnosis of cases where the RT-PCR test was either not performed at the correct time or was falsely negative. This is particularly important in individuals with delayed complications of SARS-CoV-2 infection including so-called long COVID-19 and MIS-C.[9] Although an incorrect RT-PCR result remains a possibility, this was mitigated by repeating results in house for the majority of the participants.

Limitations of this study included that symptomatology was not described in all of the positive participants. At the time of validation, the short reagent stability and lack of standardised QC material were also limitations. This has, however, been optimised by the manufacturer in more recent lots. This study does provide reassurance that, in a subset of patients, there is acceptable performance that would justify use of this test. We would recommend it is best utilised for retrospective diagnosis in individuals more than 14 days' post-positive PCR who are moderately or severely symptomatic, as an ancillary diagnostic in multisystem inflammatory disorders and seroprevalence studies.[4,27]

Acknowledgements

Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

J.G., E.M. and J.G. contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript. M.M. and T.P. contributed to design, analysis of results and to the writing of the manuscript. W.B. and J.B. contributed to design, acquisition of data and to the writing of the manuscript. S.J., L.S., W.S, A.D., M.G. and I.S. contributed to design, conception and to writing the manuscript. All authors approved final version to be published.

Funding information

Roche Diagnostics donated the kits. Sample collection was funded by the Bill and Melinda Gates Foundation (iLEAD) with grant ID: OPP1171455, and by an antiretroviral therapy simplification–optimisation of programmes and services (ART-OPS) COVID supplement from EQUIP, grant ID: AID-OAA-A-15-00070). Establishment of the in-house ELISA was supported by the Wellcome Centre for Infectious Diseases Research in Africa (CIDRI-Africa), which is supported by core funding from the Wellcome Trust [203135/Z/16/Z].

Data availability

Raw data were generated at Charlotte Maxeke Johannesburg Academic Hospital's NHLS laboratory. Derived data supporting the findings of this study are available from the corresponding author, J.G., upon reasonable request.

Disclaimer

The views expressed in the submitted article are the authors' own and not an official position of the institutions or funders.

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RESEARCH PROTOCOL

UNIVERSITY OF THE WITWATERSRAND

FACULTY OF HEALTH SCIENCES

TITLE: VALIDATION OF ROCHE IMMUNO-ASSAY FOR SEVERE ACUTE RESPIRATORY VIRUS 2 (SARS COV-2, COVID-19) IN SOUTH AFRICA

SUBMITTED BY:

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STUDENT NUMBER: 445823

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE MASTER OF MEDICINE: CHEMICAL PATHOLOGY

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1. INTRODUCTION

Coronavirus disease (COVID-19), is a viral infection caused by SARS-CoV-2, a novel coronavirus that forms part of the coronaviridae family. It was first identified in Wuhan, China, after a cluster of cases of pneumonia of unknown origin was reported in December 2019(1). COVID-19 is of zoonotic origin, which is similar to two other coronaviruses, namely SARS and MERS(2). While bats and camels were the respective intermediate hosts with the previous outbreaks, the current origin is still under investigation but bats and/or pangolins are suspected(3). It has subsequently spread to >210 countries and territories, infecting more than 6.4 million people worldwide, and caused a global pandemic, as confirmed by the WHO on 11 March 2020(4)(5).

The epidemiological burden of the disease is constantly evolving, with numbers of infected persons, hospital admissions and mortality growing on a daily basis. With a R0 (mean number of secondary cases generated by one primary case when the population is largely susceptible to infection) of 2-3, the transmission rate is higher than common seasonal respiratory viruses, which has resulted in the global “shutdown” to try and limit the spread of disease(4)(6). While conservative strategies like social distancing seem to help limit the extent of the disease(7), there is a need for rapid identification of infected individuals for the following reasons:

1. To identify asymptomatic individuals in order to isolate them and prevent further transmission(8).
2. To identify infection in at risk population groups (immunocompromised e.g. elderly, HIV, etc.) to anticipate and manage complications of COVID 19.

Until now, COVID-19 has been diagnosed with real time PCR (polymerase chain reaction) performed on a nasopharyngeal swab sample. The turnaround time for this test is 24-28 hours(9). Based on a study from Shanghai, the timeframe for deterioration in patients that developed COVID-19 complications like ARDS, was at a mean of 2.4 days after onset of symptoms(10). If the patient was not tested immediately with symptom onset, this will cause a delay with diagnosis of COVID-19, and potentially progressing to complications without knowledge of COVID-19

infection. Furthermore, this method of detection relies heavily on the presence of the viral genome in sufficient amounts at the site of sample collection that can be amplified (11).

Although RT-PCR on a nasopharyngeal or oropharyngeal sample is the gold standard, there are a few other options to detect viral presence:

a) Viral cultures, previously used as the gold standard before molecular methods were identified. It has a longer turnaround time and decreased sensitivity compared to RT-PCR, and is currently not advised for usage in COVID-19(12).
b) Detection of viral antigen also falls into this category. It is commonly used for certain other viral agents like hepatitis, usually in combination with immune markers, but it would need further developing and assessment for COVID-19 usage(13)(14).

Missing the window of viral replication as well as incorrect sampling can produce false-negative results, which can have disastrous implications for both the patient and society(15). It is therefore of significant interest to identify additional screening methods that can detect the presence of infection despite lower viral titres, and with a faster turnaround time.

There are a few additional methods to use for COVID-19 detection:

1. Radiological evidence – the chest X-ray and CT changes associated with COVID-19 has been documented as pneumonia with a ground glass appearance, as well as peri-hilar opacifications(16). Although this can be used to aid in diagnosis, the features may only present when disease progression has taken place.
2. Laboratory investigations – COVID-19 causes an increased CRP, ESR, LDH with a lymphopenia, as part of the acute phase response(10)(17). It also has an effect on electrolytes, with severe cases showing a decreased potassium and sodium(18). The pit-fall is that these abnormalities are not specific to COVID-19 infection as it is part of the acute phase response, and can thus not be used to diagnose infection.

3. Immune markers – the immune response to infection is of particular importance, since it is both specific for a pathogen and present earlier on in the disease process than radiological changes. Antibodies are rapidly produced in response to pathogens, most specifically immunoglobulin IgM markedly increases in the acute phase of infection. Although the extent and timing of the humoral response specifically against SARS-CoV-2 is still under investigation, it seems like an immune response is elicited in a delayed manner, with some individuals not showing this response at all(19). IgM and IgA antibodies seems to be detectable at median day 5, compared to IgG antibodies with a median of day 14. Of note was that a combination of PCR and IgM yielded a sensitivity of >98%(11). This has been based on small numbers, and the need for further investigations and validations exists. Furthermore, false negatives in PCR due to a decrease in viral replication as the disease progresses, can be prevented by combining with immunoglobulin studies, since this will remain positive despite lower viral loads.

It is evident that immunoglobulins will be of significant help in the fight against COVID-19. Not only can it assist with diagnosing the disease together with RT-PCR, it can also be used to establish past infections and assess immune response should there be success in developing a COVID-19 vaccine(20).

Roche has developed a method to analyse IgM and IgG antibodies on the COBAS e 602 analyser, by electrochemiluminescence immunoassay. This is done with a sandwich immunoassay method, which is thoroughly explained in Appendix A. Results are reported in a qualitative manner:

Numeric result	**Result reported**	**Interpretation**
Cut off index <1.0	Non-reactive	Negative for COVID-19 antibodies
Cut off index ≥1.0	Reactive	Positive for COVID-19 antibodies

In order to use this as a method in the laboratory, we need to confirm that results provided are both accurate and precise. Although manufacturers do extensive testing confirming that analytes

and methods conform to expectations, it is important to assess the manufacturer's claims. This can be done through a validation, which is defined as "confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled" (21).

2. STUDY OBJECTIVES

- To validate the Roche electrochemiluminescence immunoassay for COVID-19 antibody detection in South Africa.
- To assess the sensitivity of combined RT-PCR with serological testing to identify COVID-19 infection.
- To determine the sensitivity and specificity of the assay at various time points post positive PCR
- To assess the sensitivity and specificity in asymptomatic versus symptomatic patients with positive PCR.
- To describe the serologic dynamics post infection, specifically looking at the response in relation to days post positive PCR (groups described under clinical validation)

3. METHODS

STUDY DESIGN AND SETTING

A prospective analytical study that will be conducted at Charlotte Maxeke Johannesburg Academic Hospital's NHLS laboratory

PATIENT SELECTION

Patients that underwent COVID-19 testing with RT-PCR with accompanying blood in an EDTA or Lithium heparinized tube, or serum separator tube. The validation will consist of two phases:

PHASE 1: Technical validation

The technical validation will consist of using positive and negative controls. Since there is no standardized quality control (QC) material commercially available, it will be created by using three

positive pooled patient samples and five negative pooled samples as positive and negative controls respectively, as recommended by Roche (Appendix A).

Negative quality control material will be from patient samples that have tested negative for COVID-19 with RT-PCR, as well as confirmed to not have antibodies to COVID-19 via other serology methods. They will be sourced from remnant samples of patients that have tested negative for COVID 19, or remnant samples from before December 2019. Positive controls will be from patient samples that have tested positive for COVID-19, and have been confirmed to have antibodies against COVID-19. The additional serology testing that will be conducted on these quality control samples (as well as all other samples used in the validation) includes:

1. An in-house ELISA
2. A multiplexed immunofluorescence assay
3. A western blot

All samples will be tested for antibodies directed against all major structural proteins including M(embrane), E(nvelope), S(pike) and NC (nucleocapsid). In addition, all samples will also be analysed on additional formal serology platforms.

We will determine sensitivity and specificity by running 50 true positive samples that will be a mixture of low, intermediate and high titres >21 days post infection, together with 50 true negative samples(22). The titre result will be from running the samples on the in-house ELISA. The samples will be run in a blind manner; thus the result will not be known before completion of all samples.

For investigation of the reproducibility the samples will be run in duplicate. Furthermore, samples around the cut off value of 1.0 (both just below and above) will also be run in duplicate to assess precision around the cut off index.

PHASE 2: Clinical validation

This clinical validation will consist of using 350 samples of patients tested for COVID-19 with RT PCR with accompanying blood in an EDTA or lithium heparin tube or serum separating tube. The

samples will be classified on days post positive test. All samples forming part of the clinical validation will also be run on all the additional serology methods as discussed under the technical validation. Sample size:

1. Negative PCR (100 subjects)
2. Positive PCR 0-7 days post positive test (50 subjects)
3. Positive PCR 8-14 days post positive test (100 subjects)
4. Positive PCR 15-21 days post positive test (100 subjects)
5. Positive PCR > 21 days post positive test (100 subjects)

Positive samples will be sourced from: patients tested or admitted at CMJAH hospital, in collaboration with Discovery from their testing pool and from nationwide NHLS and private laboratories that have samples fitting above criteria. Negative samples will be sourced from before December 2019.

Additional data that will be collected consists of: age, gender, ethnicity, co-morbidities -specifically type II diabetes, hypertension, tuberculosis, and HIV. Furthermore the clinical history and presentation will be gathered from the “person under investigation form”, specifically: fever, shortness of breath, cough, sore throat, chills and myalgia(Appendix C). We will also look at the atypical signs and symptoms like anosmia, ageusia and dysgeusia(23). This will be analysed in conjunction with COVID-19 results.

Inclusion criteria positive samples	**Exclusion criteria positive samples**
Patients >18 years with positive COVID-19 RT-PCR	<18 years of age
	COVID-19 PCR negative
Inclusion criteria negative samples	**Exclusion criteria negative samples**
Patients > 18 years with negative COVID-19 RT-PCR	Positive COVID-19 RT-PCR
	<18 years of age

The sample size is based on the recommendation from the WHO in order to decrease the margin of error, which is greatest with a sample size of 100, but decreases when a sample size of 200-300 is used(24).

4. DATA ANALYSIS

Data will be captured on Microsoft Excel and we will use STATA for data analysis.

Results will be compared to two gold standards – RT-PCR and composite serology, which will consist of a combined result of all the additional serological methods.

Measures of diagnostic accuracy with qualitative method validations can be assessed by estimating sensitivity and specificity.

Diagnostic accuracy criteria:

	Actual results			
Method Result		*Positive*	*Negative*	*Total*
	Positive	True Positive	False Positive	TP + FP
	Negative	False Negative	True Negative	FN + TN
	Total	TP + FN	FP + TN	n

Based on above, the following calculations can be made:

Estimated sensitivity = 100 x [TP / (TP + FN)]

Estimated specificity = 100 x [TN / (FP + TN)]

(Where TN = True Negative; TP = True Positive; FN = False Negative and FP = False Positive)

The technical validation results will need to obtain a specificity of 95%, with a sensitivity of 90% in order to be acceptable(25).

We will assess the sensitivity and specificity of the test for samples in each of the four groups described above. Furthermore we will categorize participants as asymptomatic or symptomatic and assess the diagnostic sensitivity and specificity in each of these groups.

Patient demographics will be repeated as proportions, means or medians as appropriate.

Pending on the results from the validation, the assay may be included in the National Department of Health guidelines for COVID-19 testing, and used in a manner fitting with the sensitivity and specificity of the assay.

5. ETHICS

The Human Research Ethics Committee (Medical) of the University of the Witwatersrand has granted approval, clearance certificate number: M200402. The certificate is attached as Appendix B.

There are no conflicts of interest.

6. TIMING

	MAY	JUNE	JULY	AUG	SEP	OCT	NOV	DEC
1.PREPARATION								
Literature review								
Prepare protocol								
Ethics application								
Submit to MMed committee								
2. DATA COLLECTION								
Method validation								
3. DATA ANALYSIS								
4. DATA WRITE UP								
Research project								
Publication submission								
Editing and resubmission								

7. FUNDING

The method validation will be funded by Roche South Africa.

An application will be made to the NHLS for a research grant to assist with publishing costs

8. PROBLEMS

There may possibly be a lack of samples at the early stages of infection

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UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

R14/49 Dr Jurette Grove

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M200694

NAME: (Principal Investigator)	Dr Jurette Grove
DEPARTMENT:	Chemical Pathology
PROJECT TITLE:	Validation of the Roche immuno-assay for severe acute respiratory disease 2 (SARS-COV 2/COVID-19) in South Africa
DATE CONSIDERED:	06/07/2020
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISORS:	Profs' J. George and E. Mayne
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	16/07/2020

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions.Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. I **agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **July** and will therefore be due in the month of **July** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

J Grove
Principal Investigator Signature

16/07/2020
Date

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Submission ID: 1631950166
File name: Published_article_SAJID.pdf (1.13M)
Word count: 5294
Character count: 28828

Southern African Journal of Infectious Diseases
ISSN: (Online) 2313-1810, (Print) 2312-0053

Validation of Roche immunoassay for severe acute respiratory coronavirus 2 in South Africa

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Background: Serology testing is an important ancillary diagnostic to the reverse transcriptase polymerase chain reaction (RT-PCR) test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We aimed to evaluate the performance of the Roche Elecsys™ chemiluminescent immunoassay (Rotkreuz, Switzerland), that detects antibodies against the SARS-CoV-2 nucleocapsid antigen, at an academic laboratory in South Africa.

Methods: Serum samples were collected from 312 donors with confirmed positive SARS-CoV-2 RT-PCR tests, with approval from a large university's human research ethics committee. Negative controls included samples stored prior to December 2019 and from patients who tested negative for SARS-CoV-2 on RT-PCR and were confirmed negative using multiple serology methods (n = 124). Samples were stored at –80 °C and analysed on a Roche cobas™ 602 autoanalyser.

Results: Compared with RT-PCR, our evaluation revealed a specificity of 100% and overall sensitivity of 65.1%. The sensitivity in individuals > 14 days' post-diagnosis was 72.6%, with the highest sensitivity 31–50 days' post-diagnosis at 88.6%. Results were also compared with in-house serology tests that showed high agreement in majority of categories.

Conclusions: The sensitivity at all-time points post-diagnosis was lower than reported in other studies, but sensitivity in appropriate cohorts approached 90% with a high specificity. The lower sensitivity at earlier time points or in individuals without symptomatology may indicate failure to produce antibodies, which was further supported by the comparison against in-house serology tests.

Keywords: COVID-19; SARS-CoV-2; serology; antibodies; validation; immunoglobulin G; immunoglobulin M.

Introduction

The coronavirus disease 2019 (COVID-19) is a viral infection caused by severe acute respiratory coronavirus 2 (SARS-CoV-2), a novel coronavirus first identified in Wuhan, China in December 2019. It has subsequently caused a global pandemic, infecting more than 126 million people and resulting in the death of more than 2.7 million individuals worldwide.[1,2]

Whilst non-pharmacological methods such as social distancing can limit the spread of the disease, there is a need for rapid identification of infected individuals not only for diagnosis but also to prevent further transmission.[3]

The gold standard for acute SARS-CoV-2 diagnosis is the reverse transcriptase polymerase chain reaction (RT-PCR) performed on an oropharyngeal or nasopharyngeal swab sample. The acceptable turnaround time for this test in South Africa is 24 h – 48 h, but the large burden of disease and worldwide shortage of test kits have constrained availability, resulting in prolonged turnaround times worldwide.[4] Further limitations of RT-PCR include that detection relies on the presence of the viral genome in sufficient amounts for amplification.[5]

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Dates: Received: 02 Apr. 2021 | Accepted: 02 June 2021 | Published: 26 July 2021

How to cite this article: Grove JS, Mayne ES, Burgers WA, et al. Validation of Roche immunoassay for severe acute respiratory coronavirus 2 in South Africa. S Afr J Infect Dis. 2021;36(1), a286. https://doi.org/10.4102/sajid.v36i1.286

Missing the window of viral replication and incorrect sampling may produce false-negative results.[5,6] There is therefore a demand for additional testing strategies.

Serological tests that identify antibodies produced in response to infection have the potential for a rapid turnaround time.[7] Although the extent and timing of the humoral response against SARS-CoV-2 is still under investigation, immunoglobulin M (IgM) and A (IgA) antibodies directed at one or more of the major structural proteins (membrane, envelope, spike and nucleocapsid) are generally detectable at a median of day 5 and immunoglobulin G (IgG) antibodies at a median of day 14 post-symptom onset.[5] Immunoglobulin M antibody levels drop after about day 14 when the IgG antibody levels start to rise. There is potential for serological assays to be utilised in a variety of ways: to assist with diagnosis of the disease together with RT-PCR,[8] to identify past infections including in paediatric patients with multisystem inflammatory syndrome of COVID (MIS-C),[9] to perform seroprevalence studies,[4] to assess the immune response to a potential COVID-19 vaccine and, lastly, to identify donors for convalescent plasma.[10]

Since the identification of serology methods as an ancillary diagnostic, there has been a rapid development of a wide range of different assays.[4]

A review of 40 studies indicated that chemiluminescent immunoassays (CLIA) methodology had the highest sensitivity with a pooled sensitivity of 97.8%, compared with enzyme linked immunosorbent assay (ELISA) with 84.3% and lastly lateral flow immunoassays (LFIA) with 66.0%.[11] Roche Diagnostics (Rotkreuz, Switzerland) developed an electrochemiluminescent immunoassay (Elecsys™ Anti-SARS-CoV-2), which detects total antibodies against the SARS-CoV-2 nucleocapsid antigen, although it is most specific for IgG and IgM. Results are reported in a qualitative manner: below the cut-off index (COI) of 1 is interpreted as non-reactive, compared with a reactive result that is equal or greater than the COI of 1.

The manufacturer claims that this assay has a specificity of 99.81%, with a 100% sensitivity after day 14.[12] There have been multiple validations of the assay as set out in Table 1. Importantly, sensitivity is generally reported at time points postinfection of 14 days or more when IgG antibodies are more likely to be produced.

Limitations of many of these evaluations are that numbers of positive participants sampled were small and individuals tested were predominantly symptomatic. Their performance has also not been extensively examined at time points postinfection of 30 days or more raising questions regarding the persistence of an immunological response to the virus.

Of note, there have been limited validations in the African context. International studies suggest that patients of African descent are disproportionately likely to have severe disease and to die.[17] South Africa is currently the epicentre of the African pandemic with 1 545 979 cases and 52 710 deaths (29 March).[18] This prompted the evaluation of ancillary diagnostic methods in the African setting.

The auto-laboratory at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and the National Health Laboratory Service (NHLS) is a large tertiary referral laboratory servicing the Johannesburg area and surroundings. This laboratory operates a Roche cobas 602 (Rotkreuz, Switzerland). Our aim was to validate the Roche Elecsys™ electrochemiluminescent immunoassay and to assess the immune response based on symptomatology and number of days' post-molecular diagnosis and to identify the appropriate use case of this testing in South Africa.

Material and methods

Subjects

This prospective analytical study was conducted at the NHLS based at a large tertiary hospital laboratory between May and August 2020. Patients who tested positive for SARS-CoV-2 with RT-PCR on a nasopharyngeal swab within South Africa were invited to take part in the study. At the time of this study, there was a global shortage of RT-PCR reagents because of the increased demand for testing. As a result of the aforementioned problem, it was not possible to include RT-PCR results utilising only one uniform reagent. Instead, positive RT-PCR that utilised one of the following reagents were included: cobas® SARS-CoV-2 Duplex™ 2019 nCoV assay (Seegene, Korea) that targets E, ribonucleic acid-dependant polymerase (RdRP) and N genes; TaqPath™ COVID-19 V2 assay (Applied Biosystems by ThermoFisher Scientific, United States of America) that targets open reading frame of 1ab (ORF1ab), S and N genes; LightMix® Modular SARS and Wuhan CoV E-gene kit (TIB Molbiol for Roche Diagnostics, Switzerland) that targets RdRp and E genes and lastly the Abbott Alinity m SARS-CoV-2 assay (Abbott Molecular, United States) that targets N and RdRP gene. A positive RT-PCR was defined as two or more gene targets identified as positive, with a cycle threshold (Ct) of ≤ 37, as these samples were collected before

TABLE 1: Cumulative review of validations of Roche Elecsys™ severe acute respiratory virus syndrome coronavirus 2 assay.

Location	Number of positive samples included	Total number of positive participants	Number of negative controls	Reported sensitivity > 14 days post-positive RT-PCR (%)	Reported specificity (%)	Reference
Belgium	140	97	79	91.1	100.0	Favresse et al.[13]
Singapore	349	205	715	97.1	99.9	Lau et al.[14]
Germany	186	58	88	89.1	100.0	Hörber et al.
Taiwan	346	74	194	97.4 (> 21 days)	99.0	Chen et al.

RT-PCR, reverse transcriptase polymerase chain reaction.

the National Institute of Communicable Diseases' (NICD) guideline to report a positive result if one or more gene targets were identified as positive. Majority of the positive RT-PCR samples, and specifically all samples where uncertainty existed about the method that was used, had a repeat confirmatory test conducted on the same sample on the Gene Xpert (Cepheid, Sunnyvale, United States) platform within 7 days. Samples were stored for 7 days at –80 °C, and samples that could not be verified were excluded from the study. Venous blood samples were obtained after consent in a serum separator or Ethylenediaminetetraacetic acid (EDTA) tube (BD Vacutainer™) because both serum and plasma were acceptable for the platform. After centrifugation, plasma or serum was extracted, aliquoted and subsequently frozen at –80 °C, with freeze-thaw cycles limited to one. Negative control samples included remnant samples from patients stored prior to December 2019 and from patients who tested negative for SARS-CoV-2 on RT-PCR, confirmed to be negative on other serology methods, particularly in-house anti SARS-CoV-2 ELISAs, to mitigate the risk of using a false negative sample.

Data were also collected on the age and symptomatology of participants, if available and consented.

Methods

For this study we investigated the Roche Diagnostics Elecsys™ Anti-SARS-CoV-2 electrochemiluminescent immunoassay (Rotkreuz, Switzerland) that detects total antibodies against SARS-CoV-2.

Evaluation of the analytical performance was carried out in accordance with the Clinical and Laboratory Standards Institute (CLSI) EP 12 document and the United States' Food and Drug Administration (FDA) guidelines.[19,20]

Initial assay optimisation was performed on 93 patient samples prior to commencing with the full clinical validation. In the absence of standardised quality control (QC) material commercially available at the time, positive controls were derived from three positive pooled patient samples and negative controls from five negative pooled samples, as recommended by the manufacturer.

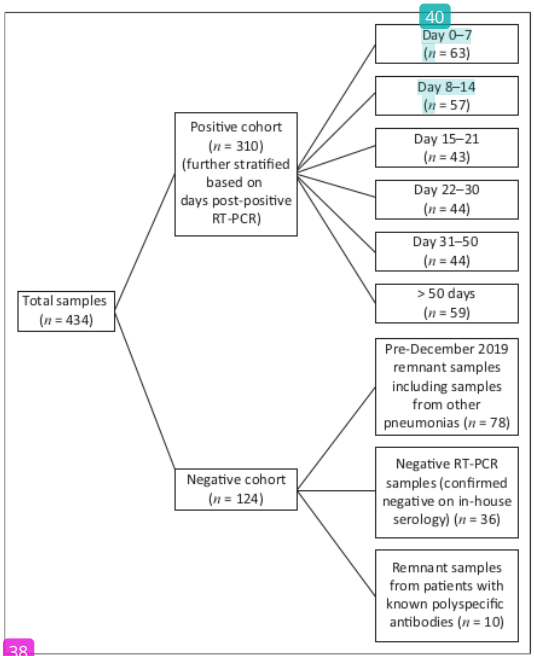
The clinical validation consisted of 434 participants' samples. This included negative samples (n = 124) and positive samples (n = 310). The positive samples were further stratified based on the number of days post RT-PCR diagnosis (Figure 1).

Samples were thawed and run on the Roche cobas™ e602 module. Although the risk of a false positive RT-PCR was mitigated by repeating analysis on the Gene Xpert platform, as discussed here, comparison against RT-PCR as the gold standard is inherently flawed because a positive RT-PCR does not guarantee a positive serology result. To address the potential misclassification of samples as false negatives, that were in fact true negatives in patients with positive RT-PCR that did not produce antibodies, a comparison was performed against a composite serology platform consisting of a Western blot and immunofluorescence, that utilised insect cell-expressed recombinant full length N and S antigens and an in-house serology ELISA assay utilising plant-based recombinant spike 1 (S1) and receptor binding domain (RBD) antigens, in collaboration with another large university.[21] After completion on the Roche platform, all samples were sent for analysis on the composite serology platform. Unfortunately, because of sample volume constraints, only 216 out of the 434 samples had sufficient results on the composite serology platform to form part of the comparison against this method. This consisted of 33 negative samples and 183 positive samples. A result was deemed as positive if 2/3 or 3/3 methods yielded a positive result.

Inter-run precision was carried out in line with the CLSI recommendation, although it had to be modified to run over 3 days instead of 5 because the on-board stability of the reagent at the time of validation was only 72 h.

Statistical analysis

Results were compared with both the RT-PCR and disaggregated by days post-diagnosis. Samples were further stratified by symptom score, which was assigned as follows: 0 for asymptomatic, 1 for mild diseases indicating only respiratory tract symptoms, 2 for moderate disease indicating symptoms outside of the respiratory tract and 3 for severe disease requiring admission. Samples were further compared with in-house serology, to assess the presence of detectable



RT-PCR, reverse transcriptase polymerase chain reaction.

FIGURE 1: Summary of samples used in validation.

TABLE 2: Sensitivity of stratified data – Days post positive reverse transcriptase polymerase chain reaction and symptoms, compared to reverse transcriptase polymerase chain reaction and agreement compared with composite serology.

Stratified data days post positive RT-PCR	Sensitivity compared to RT-PCR (%)	95% confidence interval (%)	Agreement compared with composite serology (%)	95 % confidence interval (%)
0–7 days	52.4	39.4–65.1	84.6	54.6–98.1
8–14 days	54.4	40.7–67.6	77.8	40–97.2
15–21 days	72.1	56.3–84.7	88.2	63.6–98.5
22–30 days	47.7	32.5–63.3	66.7	34.9–90.1
31–50 days	88.6	75.4–96.2	100.0	86.3–100
> 14 days	72.6	65.7–78.8	93.3	85.9–97.5
> 50 days	79.7	75.4–96.2	100.0	86.3–100
Symptom score				
0 (asymptomatic)	57.6	39.2–74.5	81.8	48.2–97.7
1 (mildly symptomatic)	59.2	44.2–73	82.6	61.2–95
2 (moderately symptomatic)	64.4	53.7–74.3	91.7	80–97.7
3 (severely symptomatic)	69.8	57–80.7	90.9	58.7–99.8
Stratified data – Days post-PCR in symptomatic individuals				
> 14 days in moderately to severely symptomatic	81	70.6–89	97.6	87.4–99.9

RT-PCR, reverse transcriptase polymerase chain reaction.

TABLE 3: Sensitivity with the asymptomatic cohort removed.

Stratified data days post positive RT-PCR (days)	Sensitivity compared with RT-PCR without the asymptomatic cohort (%)	95% confidence interval (%)
0–7	47.6	32–63.6
8–14	50	34.2–65.8
15–21	85.7	67.3–95.8
22–30	61.3	42.2–78.2
31–50	90	73.5–97.9
> 14	76.5	67.7–83.9
	70	48.2–85.7

RT-PCR, reverse transcriptase polymerase chain reaction.

antibodies of results because the limitations of RT-PCR are well known (including the ability to detect past infection).

Data analysis

Statistical analysis was performed with MedCalc statistical software version 19.4.1 (MedCalc Software Ltd., Belgium). We defined sensitivity as the proportion of correctly identified COVID-19-positive patients who were positive by RT-PCR SARS-CoV-2 analysis in respiratory samples, whilst specificity was defined as the proportion of correctly identified negative samples. Sensitivity was also reported according to symptom score and days post positive RT-PCR.

Inter-run precision was calculated with Microsoft Excel as CV (%) = (standard deviation [SD] × 100)/mean, and expressed as a percentage coefficient of variation.

Ethical considerations

Ethical approval to conduct the study was obtained from the Human Research Ethics Council of the University of the Witwatersrand (reference number: M200694). Informed consent was obtained from all participants.

Results

The median age of both cohorts was 42, the positive cohort (n = 310) with a range of 19–87 years (minimum to maximum), compared with the negative control (n = 124) of 22–75 years. Both cohorts had a slight female preponderance (61.6% in the positive group and 57.1% in the negative group). By symptom category, moderately symptomatic participants were the largest group (n = 90) and asymptomatic participants were the smallest group (n = 33). Participants were almost equally distributed by number of days post diagnosis, except for those between 0 and 7 days who made up 20% of the total cohort (n = 63).

Accuracy analysis

The specificity of the assay was high at 100% (95% confidence interval [CI] 97.07% – 100%). The overall sensitivity of the Roche assay across all participants was 65.2% (95% CI 59.57% – 70.46%) when compared with RT-PCR results.

Samples were also stratified by days post RT-PCR and degree of symptoms and compared with both the RT-PCR and the in-house composite serology platform (Table 2). Comparison against RT-PCR demonstrated that sensitivity was greatest at > 14 days post PCR in severely symptomatic participants. Comparison against in-house serology demonstrated much higher agreement in all groups, with 100% > 30 days post positive RT-PCR.

Compared with the in-house serology, the overall positive agreement was 89.4% (95% CI 82.18% – 94.39%), with a slight reduction in negative agreement of 88.4% (95% CI 80.53% – 93.83%). The asymptomatic group demonstrated the lowest sensitivity or agreement across both comparisons.

As the asymptomatic group revealed the lowest sensitivity, analysis of the data was repeated with removal of asymptomatic patients from all cohorts, which revealed an increase in the sensitivity in all the groups > 14 days, apart from a slight decline in > 50 days, as set out in Table 3.

Precision analysis

In order to assess inter-run repeatability, 98 of the samples were tested in duplicate, with a qualitative repeatability result

of 100%. Ten samples with results around the COI of one were identified and also tested in duplicate, with 100% agreement.

The QC material, made up as recommended by the manufacturer, was tested five times over 3 days to accommodate the short on-board reagent stability. The assay showed acceptable precision with an index value of 0.093 and a coefficient of variation of 1.5% in the negative controls, and an index value of 3.14 and a coefficient of variation (CV) of 2.2% in the positive controls. This broadly agrees with the % CV of other immunoassays performed on the cobas e602.[14,22]

Samples from patients with known autoimmune disease and poly-specific immunoglobulins, obtained before February 2020, were also analysed (n = 10), with 100% specificity. Seven of these samples were tested in duplicate, with 100% result concordance.

Discussion

This study aimed to evaluate the test performance of the Roche Elecsys Anti-SARS-CoV 2 assay tested on a cobas 602 in a high prevalence setting in South Africa. Results were compared with RT-PCR as the gold standard. This revealed a variable sensitivity ranging from 47.8% to 88.6% and a high specificity of 100%, the latter in line with the manufacturer's claims.[12]

These findings support the use of this assay for the case proposed including for retrospective diagnosis and for seroprevalence studies because a high diagnostic specificity is essential in these settings. There was no cross-reactivity observed with samples that had poly-specific antibodies. Sensitivity was moderately low in individuals more than 14 days' post-positive test result at 72.63%, but improved in patients with moderate or severe symptoms. This test demonstrated the highest sensitivity compared with RT-PCR in severely ill or admitted individuals at time points of 14 days post-diagnosis although this failed to reach a level of 100% sensitivity after 14 days in any group as claimed by the manufacturer.[12] This study found low sensitivity of the assay in asymptomatic patients and in patients before 14 days post-diagnosis, which agrees with findings from other validations internationally. This reflects the dynamics of the humoral response to SARS-CoV-2, which suggests that IgG antibodies are only detectable on day 10 post-infection and reach a maximum after day 14 and IgM antibodies are produced at about day 5–7 but are transient and are not produced by all patients.[5,13,15,23] This is further supported by the high agreement in these time points compared with composite serology. There was a slight decline in sensitivity seen at day 50, but this was insignificant. Although few studies of antibody persistence have been published, there are suggestions in some cases that antibody levels may decline after 2 months and that IgM particularly is undetectable after 30–60 days in most patients.[24,25] The poor performance in day 22–30 post diagnosis could not be fully explained, although only a third of the patients with known symptomatology were classified as severe, which may have skewed the data in this participant group. This was supported by the increase in sensitivity seen after reanalysis without the asymptomatic patients in the group, although an important confounder remained that symptomatology was not known for a fifth of the group. Importantly, sensitivity remained low when compared with the in-house ELISA and the possibility exists that this indicates that anti-N antibodies were not produced in this subset.

Previous studies have shown that antibodies against the N- and S-protein are produced more or less at the same time, however there is a need for further data to evaluate if antibodies against both are produced in all subjects.[23]

This study is the most extensive evaluation of the Roche Elecsys Anti SARS-CoV-2 electrochemiluminescence immunoassay, assessing all time points post-positive RT-PCR, but most specifically more than 21 days post-positive diagnosis and correlating results with symptomatology. The assay had a high specificity in line with global validations and manufacturer claims. This validation showed the lowest sensitivity in the asymptomatic symptom group, which seems to confirm that not all individuals infected with SARS-CoV-2 produce systemic antibodies, especially if asymptomatic.[26] Cumulative sensitivity and sensitivities at all the time points compared with RT-PCR as gold standard, were lower than other comparable studies reported globally, but the inclusion of asymptomatic participants in all groups may offer a partial explanation for this decline.[13,14,15,16] The agreement in all groups significantly improved with comparison against in-house serology. Importantly, the viral and humoral response dynamics indicate that these two assays are sensitive at different time points in infection with the RT-PCR more sensitive prior to day 14 and the serology more sensitive thereafter. This supports the use of these tests for the indications that are approved in South Africa including for retrospective diagnosis of cases where the RT-PCR test was either not performed at the correct time or was falsely negative. This is particularly important in individuals with delayed complications of SARS-CoV-2 infection including so-called long COVID-19 and MIS-C.[9] Although an incorrect RT-PCR result remains a possibility, this was mitigated by repeating results in house for the majority of the participants.

Limitations of this study included that symptomatology was not described in all of the positive participants. At the time of validation, the short reagent stability and lack of standardised QC material were also limitations. This has, however, been optimised by the manufacturer in more recent lots. This study does provide reassurance that, in a subset of patients, there is acceptable performance that would justify use of this test. We would recommend it is best utilised for retrospective diagnosis in individuals more than 14 days' post-positive PCR who are moderately or severely symptomatic, as an ancillary diagnostic in multisystem inflammatory disorders and seroprevalence studies.[4,27]

Acknowledgements

Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

J.G., E.M. and J.G. contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript. M.M. and T.P. contributed to design, analysis of results and to the writing of the manuscript. W.B. and J.B. contributed to design, acquisition of data and to the writing of the manuscript. S.J., L.S., W.S, A.D., M.G. and [illegible] contributed to design, conception and to writing the manuscript. All authors approved final version to be published.

Funding information

Roche Diagnostics donated the kits. Sample collection was funded by the Bill and Melinda Gates Foundation (iLEAD) with grant ID: OPP1171455, and by an antiretroviral therapy simplification–optimisation of programmes and services (ART-OPS) COVID supplement from EQUIP, grant ID: AID-OAA-A-15-00070). Establishment of the in-house ELISA was supported by the Wellcome Centre for Infectious Diseases Research in Africa (CIDRI-Africa), which is supported by core funding from the Wellcome Trust [203135/Z/16/Z].

Data availability

Raw data were generated at Charlotte Maxeke Johannesburg Academic Hospital's NHLS laboratory. Derived data supporting the findings of this study are available from the corresponding author, J.G., upon reasonable request.

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Validation of Roche immunoassay for severe acute respiratory virus 2/SARS-COV-2 in South Africa

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ABSTRACT

Background

Serology testing is an important ancillary diagnostic to the reverse transcriptase polymerase chain reaction (RT-PCR) test for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). We aimed to evaluate the performance of the Roche Elecsys™ chemiluminescent immunoassay (Rotkreuz, Switzerland), that detects antibodies against the SARS-CoV-2 nucleocapsid antigen, at an academic laboratory in South Africa.

Methods

Serum samples were collected from 312 donors with confirmed positive SARS-CoV-2 RT-PCR tests, with approval from a large university's human research ethics committee. Negative controls included samples stored prior to December 2019, and from patients that tested negative for SARS-CoV-2 on RT-PCR and were confirmed negative using multiple serology methods (n=124). Samples were stored at -80ºC and analysed on a Roche cobas™ 602 autoanalyzer.

Results

Compared to RT-PCR, our evaluation revealed a specificity of 100% and overall sensitivity of 65.1%. The sensitivity in individuals >14 days' post diagnosis was 72.6%, with the highest sensitivity 31-50 days' post-diagnosis at 88.6%. Results were also compared to in-house serology tests that showed high agreement in majority of categories.

Conclusions

The sensitivity at all-time points post-diagnosis was lower than reported in other studies, but sensitivity in appropriate cohorts approached 90% with a high specificity. The lower sensitivity at earlier time points or in individuals without symptomatology may indicate failure to produce antibodies, which was further supported by the comparison against in-house serology tests.

KEYWORDS

COVID-19, SARS-CoV-2, serology, antibodies, validation, immunoglobulin G, immunoglobulin M.

INTRODUCTION

Coronavirus disease (COVID-19) is a viral infection caused by severe acute respiratory coronavirus 2 (SARS-CoV-2), a novel coronavirus first identified in Wuhan, China in December 2019. It has subsequently caused a global pandemic, infecting more than 126 million people and resulting in the death of more than 2.7 million individuals worldwide (1,2).

While non-pharmacological methods like social distancing can limit the spread of the disease, there is a need for rapid identification of infected individuals, not only for diagnosis, but also to prevent further transmission(3).

The gold standard for acute SARS-CoV-2 diagnosis is the reverse transcriptase polymerase chain reaction (RT-PCR) performed on an oropharyngeal or nasopharyngeal swab sample. The acceptable turnaround time for this test in South Africa is 24-48 hours, but the large burden of disease and world-wide shortage of test kits have constrained availability, resulting in prolonged turnaround times worldwide(4). Further limitations of RT-PCR include that detection relies on the presence of the viral genome in sufficient amounts for amplification(5).

Missing the window of viral replication as well as incorrect sampling may produce false-negative results(5,6). There is therefore a demand for additional testing strategies.

Serological tests which identify antibodies produced in response to infection have the potential for a rapid turn-around time(7). Although the extent and timing of the humoral response against SARS-CoV-2 is still under investigation, immunoglobulin M (IgM) and A (IgA) antibodies directed at one or more of the major structural proteins (membrane, envelope, spike and nucleocapsid) are generally detectable at a median of day 5, and immunoglobulin G (IgG) antibodies at a median of day 14 post-symptom onset(5). IgM antibody levels drop after about day 14 when the IgG antibody levels start to rise. There is potential for serological assays to be utilized in a variety of ways: to assist with diagnosis of the disease together with RT-PCR(8), to identify past infections including in paediatric patients with multisystem inflammatory

syndrome of COVID (MIS-C)(9), to perform sero-prevalence studies(4), to assess the immune response to a potential COVID-19 vaccine and, lastly, to identify donors for convalescent plasma(10).

Since the identification of serology methods as an ancillary diagnostic, there has been a rapid development of a wide range of different assays(4).

A review of 40 studies indicated that chemiluminescent immuno-assays (CLIA) methodology had the highest sensitivity with a pooled sensitivity of 97.8%, compared to enzyme linked immunosorbent assay (ELISA) with 84.3%, and lastly lateral flow immuno-assays (LFIA) with 66.0%(11). Roche Diagnostics (Rotkreuz, Switzerland) developed an electrochemiluminescent immunoassay (Elecsys™ Anti-SARS-CoV-2), which detects total antibodies against the SARS-CoV-2 nucleocapsid antigen, although it is most specific for IgG and IgM. Results are reported in a qualitative manner: below the cut-off index (COI) of 1 is interpreted as non-reactive, compared to a reactive result that is equal or greater than the COI of 1.

The manufacturer claims that this assay has a specificity of 99.81%, with a 100% sensitivity after day 14(12). There have been multiple validations of the assay as set out in table 1. Importantly, sensitivity is generally reported at time points post infection of 14 days or more when IgG antibodies are more likely to be produced.

Table 1: Cumulative review of validations of Roche Elecsys™ SARS-CoV-2 assay

Location	Number of positive samples included	Total number of positive participants	Number of negative controls	Reported sensitivity >14 days post positive qPCR	Reported specificity	Reference
Belgium	140	97	79	91.1%	100%	(13)
Singapore	349	205	715	97.1%	99.9%	(14)
Germany	186	58	88	89.1%	100%	(15)
Taiwan	346	74	194	97.4% (>21 days)	99.0%	(16)

Limitations of many of these evaluations are that numbers of positive participants sampled were small and individuals tested were predominantly symptomatic. Their performance has also not been extensively examined at time points post infection of 30 days or more raising questions regarding the persistence of an immunological response to the virus.

Of note, there have been limited validations in the African context. International studies suggest that patients of African descent are disproportionately likely to have severe disease and to die(17). South Africa is currently the epicentre of the African pandemic with 1 545 979 cases and 52 710 deaths (29 March)(18). This prompted the evaluation of ancillary diagnostic methods in the African setting.

The auto-laboratory at (redacted for review purposes) and the National Health Laboratory Service (NHLS) is a large tertiary referral laboratory servicing the (redacted for review purposes) area and surrounds. This laboratory operates a Roche cobas 602 (Rotkreuz, Switzerland). Our aim was to validate the Roche Elecsys™ electrochemiluminescent immunoassay and to assess the immune response based on symptomatology, as well as number of days' post molecular diagnosis, and to identify the appropriate use case of this testing in South Africa.

MATERIAL AND METHODS

Ethics: Ethical clearance was obtained from the Human Research Ethics Committee of [information redacted to maintain the integrity of the review process]. Ethical clearance was obtained for the in-house serology from the Human Research Ethics Committee of [information redacted to maintain the integrity of the review process], against which this study's results were compared.

Subjects: This prospective analytical study was conducted at the NHLS based at a large tertiary hospital's laboratory between May and August 2020. Patients that tested positive for SARS-CoV-2 with RT-PCR on a nasopharyngeal swab within South Africa were invited to take part in the study. At the time of this study, there was a global shortage of RT-PCR reagents due to the increased demand for testing. Due to the aforementioned problem, it was not possible to include RT-PCR results utilizing only one uniform reagent. Instead, positive RT-PCR that

utilized one of the following reagents were included: Allplex™ 2019 nCoV assay (Seegene, Korea) that targets E, RNA-dependant polymerase (RdRP) and N genes; TaqPath™ COVID-19 V2 assay (Applied Biosystems by ThermoFisher Scientific, United States) that targets ORF1ab, S and N genes; LightMix® Modular SARS and Wuhan CoV E-gene kit (TIB Molbiol for Roche Diagnostics, Switzerland) that targets RdRp and E genes, and lastly the Abbott Alinity m SARS-CoV-2 assay (Abbott Molecular, United States) that targets N and RdRP gene [R1.1]. A positive RT-PCR was defined as two or more gene targets identified as positive, with a cycle threshold (Ct) of ≤37, as these samples were collected before the National Institute of Communicable Diseases' (NICD) guideline to report a positive result if one or more gene targets were identified as positive. Majority of the positive RT-PCR samples, and specifically all samples where uncertainty existed about the method that was used, had a repeat confirmatory test conducted on the same sample on the Gene Xpert (Cepheid, Sunnyvale, CA) platform within 7 days. Samples were stored for 7 days at -80 degrees Celsius, and samples that could not be verified were excluded from the study. Venous blood samples were obtained after consent in a serum separator or EDTA tube (BD Vacutainer™), since both serum and plasma were acceptable for the platform. After centrifugation, plasma or serum was extracted, aliquoted, and subsequently frozen at -80^{0}C, with freeze-thaw cycles limited to one. Negative control samples included remnant samples from patients stored prior to December 2019 and from patients that tested negative for SARS-CoV-2 on RT-PCR, confirmed to be negative on other serology methods, particularly in-house anti SARS-CoV-2 ELISAs, to mitigate the risk of using a false negative sample.

Data were also collected on the age and symptomatology of participants, if available and consented.

Methods:

For this study we investigated the Roche Diagnostics Elecsys™ Anti-SARS-CoV-2 electrochemiluminescent immunoassay (Rotkreuz, Switzerland), that detects total antibodies against SARS-CoV-2.

Evaluation of the analytical performance was carried out in accordance with the Clinical and Laboratory Standards Institute (CLSI) EP 12 document, as well as the United States' Food and Drug Administration (FDA) guidelines(19)(20).

Initial assay optimisation was performed on 93 patient samples prior to commencing with the full clinical validation. In the absence of standardized quality control (QC) material commercially available at the time, positive controls were derived from three positive pooled patient samples and negative controls from five negative pooled samples, as recommended by the manufacturer.

The clinical validation consisted of 434 participants' samples. This included negative samples (n=124) and positive samples (n=310). The positive samples were further stratified based on the number of days post RT-PCR diagnosis. (Figure 1).

Samples were thawed and run on the Roche cobas™ e602 module. Although the risk of a false positive RT-PCR was mitigated by repeating analysis on the Gene Xpert platform, as discussed above, comparison against RT-PCR as the gold standard is inherently flawed since a positive RT-PCR does not guarantee a positive serology result. To address the potential misclassification of samples as false negatives, that were in fact true negatives in patients with positive RT-PCR that did not produce antibodies, a comparison was done against a composite serology platform consisting of a Western blot and immunofluorescence, that utilised insect cell-expressed recombinant full length N and S antigens, as well as an in-house serology ELISA assay utilising plant-based recombinant S1 and RBD antigens, in collaboration with another large university(21). After completion on the Roche platform, all samples were sent for analysis on the composite serology platform. Unfortunately, due to sample volume constraints, only 216 out of the 434 samples had sufficient results on the composite serology platform to form part of the comparison against this method. This consisted of 33 negative samples and 183 positive samples. A result was deemed as positive if 2/3 or 3/3 methods yielded a positive result.

Inter-run precision was carried out in line with the CLSI recommendation, although it had to be modified to run over 3 days instead of 5, since the on-board stability of the reagent at the time of validation was only 72 hours.

190 Figure 1: Summary of samples used in validation:

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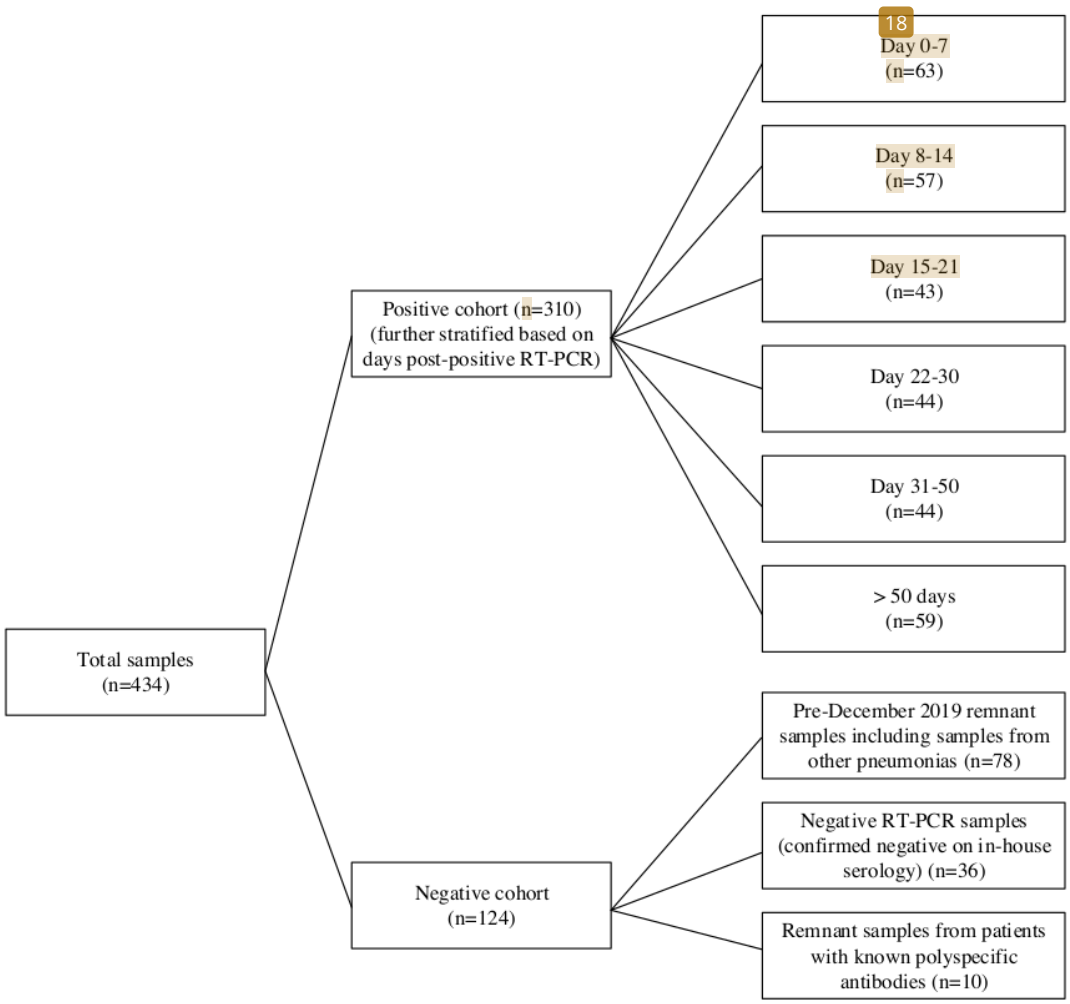

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Statistical analysis:

Results were compared to both the RT-PCR and disaggregated by days post-diagnosis. Samples were further stratified by symptom score, which was assigned as follows: 0 for asymptomatic, 1 for mild diseases indicating only respiratory tract symptoms, 2 for moderate disease indicating symptoms outside of the respiratory tract and 3 for severe disease requiring admission. Samples were further compared to in-house serology, to assess the presence of detectable antibodies of results, since the limitations of RT-PCR are well known (including the ability to detect past infection).

Data analysis:

Statistical analysis was performed with MedCalc statistical software version 19.4.1 (MedCalc Software Ltd, Ostend, Belgium). We defined sensitivity as the proportion of correctly identified COVID-19-positive patients who were positive by RT-PCR SARS-CoV-2 analysis in respiratory samples, while specificity was defined as the proportion of correctly identified negative samples. Sensitivity was also reported according to symptom score and days post positive RT-PCR.

Inter-run precision was calculated with Microsoft Excel as CV (%) = (SD×100)/ mean, and expressed as a percentage coefficient of variation.

RESULTS

The median age of both cohorts was 42, the positive cohort (n=310) with a range of 19-87 years (minimum to maximum), compared to the negative control (n=124) of 22-75 years. Both cohorts had a slight female preponderance (61.6% in the positive group and 57.1% in the negative group). By symptom category, moderately symptomatic participants were the largest group (n=90), and asymptomatic participants were the smallest group (n=33), Participants were almost equally distributed by number of days post diagnosis, except for those between 0-7 days who made up 20% of the total cohort (n=63).

Accuracy analysis

The specificity of the assay was high at 100% (95% CI 97.07-100%). The overall sensitivity of the Roche assay across all participants was 65.2% (95% CI 59.57-70.46%) when compared with RT-PCR results.

Samples were also stratified by days post RT-PCR and degree of symptoms and compared with both the RT-PCR and the in-house composite serology platform (Table 3). Comparison against RT-PCR demonstrated that sensitivity was greatest at > 14 days post PCR in severely symptomatic participants. Comparison against in-house serology demonstrated much higher agreement in all groups, with 100% > 30 days post positive RT-PCR.

Compared to the in-house serology, the overall positive agreement was 89.4% (95% CI 82.18%-94.39%), with a slight reduction in negative agreement of 88.4% (95% CI 80.53%-93.83%). The asymptomatic group demonstrated the lowest sensitivity or agreement across both comparisons.

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244 Table 3: Sensitivity of stratified data – days post positive RT-PCR and symptoms, compared
245 to RT-PCR and agreement compared to composite serology.

Stratified data days post positive RT-PCR	Sensitivity compared to RT-PCR	Agreement compared to composite serology
0-7 days	52.4% (39.4%-65.1%)	84.6% (54.6% - 98.1%)
8-14 days	54.4% (40.7%-67.6%)	77.8% (40% - 97.2%)
15-21 days	72.1% (56.3%-84.7%)	88.2% (63.6% - 98.5%)
22-30 days	47.7% (32.5%-63.3%)	66.7% (34.9% - 90.1%)
31-50 days	88.6% (75.4%-96.2%)	100.0% (86.3% - 100%)
> 14 days	72.6% (65.7%-78.8%)	93.3% (85.9% - 97.5%)
> 50 days	79.7% (75.4%-96.2%)	100.0% (86.3% - 100%)
Symptom score		
0 (asymptomatic)	57.6% (39.2%-74.5%)	81.8% (48.2% - 97.7%)
1 (mildly symptomatic)	59.2% (44.2%-73%)	82.6% (61.2% - 95%)
2 (moderately symptomatic)	64.4% (53.7%-74.3%)	91.7% (80% - 97.7%)
3 (severely symptomatic)	69.8% (57%-80.7%)	90.9% (58.7% - 99.8%)
Stratified data – days post-PCR in symptomatic individuals		
> 14 days in moderately to severely symptomatic	81% (70.6%-89%)	97.6% (87.4%-99.9%)

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247 As the asymptomatic group revealed the lowest sensitivity, analysis of the data was repeated
248 with removal of asymptomatic patients from all cohorts, which revealed an increase in the
249 sensitivity in all the groups > 14 days, apart from a slight decline in >50 days, as set out in table
250 4.

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252

253 Table 4: Sensitivity with the asymptomatic cohort removed:

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Stratified data days post positive RT-PCR	Sensitivity compared to RT-PCR without the asymptomatic cohort
0-7 days	47.6% (32% – 63.6%)
8-14 days	50% (34.2%-65.8%)
15-21 days	85.7% (67.3%-95.8%)
22-30 days	61.3% (42.2%-78.2%)
31-50 days	90% (73.5%-97.9%)
> 14 days	76.5% (67.7%-83.9%)
> 50 days	70% (48.2%-85.7%)

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Precision analysis

In order to assess inter-run repeatability, 98 of the samples were tested in duplicate, with a qualitative repeatability result of 100%. 10 samples with results around the COI of 1 were identified and also tested in duplicate, with 100% agreement.

The quality control material, made up as recommended by the manufacturer, was tested 5 times over 3 days to accommodate the short on-board reagent stability. The assay showed acceptable precision with an index value of 0.093 and a coefficient of variation of 1.5% in the negative controls, and an index value of 3.14 and a coefficient of variation of 2.2% in the positive controls. This broadly agrees with the %CV of other immunoassays performed on the cobas e602(14,22).

Samples from patients with known autoimmune disease and poly-specific immunoglobulins, obtained before February 2020, were also analysed (n=10), with 100% specificity. Seven of these samples were tested in duplicate, with 100% result concordance.

DISCUSSION

This study aimed to evaluate the test performance of the Roche Elecsys Anti-SARS-CoV2 assay tested on a cobas 602 in a high prevalence setting in South Africa. Results were compared to RT-PCR as the gold standard. This revealed a variable sensitivity ranging from 47.8%-88.6% and a high specificity of 100%, the latter in line with the manufacturer's claims(12).

These findings support the use of this assay for the case proposed including for retrospective diagnosis and for sero-prevalence studies, since a high diagnostic specificity is essential in these settings. There was no cross-reactivity observed with samples that had poly-specific antibodies. Sensitivity was moderately low in individuals more than 14 days' post-positive test result at 72.63%, but improved in patients with moderate or severe symptoms. This test demonstrated the highest sensitivity compared with RT-PCR in severely ill or admitted individuals at time points of 14 days post-diagnosis although this failed to reach a level of 100% sensitivity after 14 days in any group as claimed by the manufacturer(12). This study found low sensitivity of the assay in asymptomatic patients and in patients before 14 days post-

diagnosis which agrees with findings from other validations internationally. This reflects the dynamics of the humoral response to SARS-CoV-2 which suggests that IgG antibodies are only detectable at about day 10 post-infection and reach a maximum after day 14, and IgM antibodies are produced at about day 5-7 but are transient and are not produced by all patients(5,13,15,23). This is further supported by the high agreement in these time points compared to composite serology. There was a slight decline in sensitivity seen at day 50, but this was insignificant. Although few studies of antibody persistence have been published, there are suggestions in some cases that antibody levels may decline after 2 months and that IgM particularly is undetectable after 30-60 days in most patients(24,25). The poor performance in day 22-30 post diagnosis could not be fully explained, although only a third of the patients with known symptomatology were classified as severe, which may have skewed the data in this participant group. This was supported by the increase in sensitivity seen after reanalysis without the asymptomatic patients in the group, although an important confounder remained that symptomatology was not known for a fifth of the group. Importantly, sensitivity remained low when compared with the in-house ELISA and the possibility exists that this indicates that anti-N antibodies were not produced in this subset.

Previous studies have shown that antibodies against the N- and S-protein are produced more or less at the same time, however there is a need for further data to evaluate if antibodies against both are produced in all subjects(23).

This study is the most extensive evaluation of the Roche Elecsys Anti SARS-CoV-2 electrochemiluminescence immuno-assay, assessing all time points post positive RT-PCR, but most specifically more than 21 days post positive diagnosis, as well as correlating results with symptomatology. The assay had a high specificity in line with global validations and manufacturer claims. This validation showed the lowest sensitivity in the asymptomatic symptom group, which seems to confirm that not all individuals infected with SARS-CoV-2 produce systemic antibodies, especially if asymptomatic(26). Cumulative sensitivity and sensitivities at all the time points compared to RT-PCR as gold standard, were lower than other comparable studies reported globally, but the inclusion of asymptomatic participants in all groups may offer a partial explanation for this decline(13–16). The agreement in all groups significantly improved with comparison against in-house serology. Importantly, the viral and humoral response dynamics indicate that these two assays are sensitive at different time points

in infection with the RT-PCR more sensitive prior to day 14 and the serology more sensitive thereafter. This supports the use of these tests for the indications that are approved in South Africa including for retrospective diagnosis of cases where the RT-PCR test was either not performed at the correct time or was falsely negative. This is particularly important in individuals with delayed complications of SARS-CoV-2 infection including so-called long COVID-19 and MIS-C(9). Although an incorrect RT-PCR result remains a possibility, this was mitigated by repeating results in house for the majority of the participants.

Limitations of this study included that symptomatology was not described in all of the positive participants. At the time of validation, the short reagent stability and lack of standardized quality control material were also limitations. This has, however, been optimised by the manufacturer in more recent lots. This study does provide reassurance that, in a subset of patients, there is acceptable performance that would justify use of this test. We would recommend it is best utilised for retrospective diagnosis in individuals more than 14 days' post positive PCR who are moderately or severely symptomatic, as an ancillary diagnostic in multi-system inflammatory disorders and sero-prevalence studies(4,27).

ACKNOWLEDGEMENTS AND STATEMENTS

Acknowledgements: None

Competing interests: The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Author contributions: [information redacted to maintain the integrity of the review process]

Funding information: [information redacted to maintain the integrity of the review process]

Data availability statement: Raw data were generated at (redacted for review purposes). Derived data supporting the findings of this study are available from the corresponding author on request.

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