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ORIGINAL ARTICLE

Method Comparison of two different Serological Assays for Detection of Antibodies against SARS-COV-2 in Vaccinated Individuals

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ABSTRACT

Background: Public health has been greatly damaged by the worldwide Coronavirus Disease 2019 (COVID-19) outbreak, which was brought on by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Strong diagnostic instruments are now required in order to precisely identify and track immunity due to the pandemic.

Objective: This study aimed to compare the performance of two serological assays in detecting antibodies against SARS-CoV-2 in vaccinated individuals.

Methods: From August 1, 2023, to January 31, 2024, a cross-sectional study was carried out at the Dow International Medical College in Karachi, in the Section of Chemical Pathology. Analysis was done on a convenience sample of 187 laboratory employees that was non-probability. Siemens Healthineers Atellica® IM SARS-CoV-2 IgG and ROCHE Elecsys® were used to evaluate blood samples. Software for SPSS and R was used to conduct statistical studies, which included Bland-Altman plots, bivariate regression analysis, descriptive statistics, Wilcoxon signed-rank test, and Passing-Bablok regression. There was a significance threshold of $p < 0.05$

Results: Among all participants, 48.7% and 51.3% were tested reactive for Siemens, and Roche, respectively. Bivariate regression analyses showed weak correlations for age, gender, Covid-19 status, and vaccination status with both assays. The Bland-Altman plot demonstrated good concordance (red line at 0) between Siemens and Roche assays, though a few outliers were noted. Passing-Bablok regression analysis revealed a proportional relationship with Roche values generally higher than Siemens, but with moderate correlation.

Conclusion: Both Siemens and Roche assays are reliable for detecting SARS-CoV-2 antibodies, with Roche showing slightly higher values. The findings highlight the utility of serological testing in complementing molecular diagnostics and monitoring immune responses in vaccinated individuals.

Keywords: Serological assays, antibody detection, vaccination, immune response, SARS-CoV-2

INTRODUCTION

Global public health faced an unprecedented challenge with the advent of the Coronavirus Disease 2019 (Covid-19), which was caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The virus, which began in Wuhan, China, in late 2019, quickly spread throughout the world, prompting the World Health Organization (WHO) to declare a pandemic on March 11, 2020 [1, 2]. As of July 14, 2024, there have been over 776 million COVID-19 cases registered to the WHO worldwide, with about 1.6 million of those instances occurring in Pakistan [3].

SARS-CoV-2 was a member of the beta coronavirus genus and had genetic characteristics with SARS-CoV and MERS-CoV, two additional coronaviruses that caused human illness. The single-stranded RNA (+ssRNA) that makes up the genomic structure of the virus encodes a number of structural proteins, chief among them the spike (S) glycoprotein. This S glycoprotein is an essential target for both therapeutic and diagnostic approaches because it bound to the angiotensin-converting enzyme 2 (ACE2) receptor, facilitating viral entrance into host cells [4].

The focus during the early stages of the pandemic was on using molecular techniques, like as real-time reverse transcriptase-polymerase chain reaction (RT-PCR), to detect viral RNA. [5]. This method remained the gold standard for diagnosing active infections due to its high sensitivity and specificity [5,6]. However, the reliance on RT-PCR highlighted limitations, particularly as viral RNA levels could diminish over the course of the infection, potentially leading to false negatives, especially in individuals with mild or asymptomatic cases [7,8].

To address these gaps, serological assays were developed to detect SARS-CoV-2. These assays provided critical insights into the immune response, epidemiology, and potential immunity within populations. Antibody-based surveillance complemented molecular diagnostics by identifying individuals who had been exposed to the virus [9].

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Numerous serological assays were developed, including chemiluminescent immunoassays (CLIAs), lateral flow immunoassays (LFIAs), and enzyme-linked immunosorbent assays (ELISAs) [10]. Antibodies directed against the highly immunogenic spike (S) and nucleocapsid (N) proteins of SARS-CoV-2 were the main focus of these investigations. The S protein's function in viral entry and as a target for neutralizing antibodies made it, in particular, its receptor-binding domain (RBD), of great interest. [11].

The performance of serological assays varied, with specificities generally exceeding 95% but sensitivities ranging between 70% and 90%, depending on the population and timing of sample collection. Sensitivity tended to peak approximately two weeks post-infection, aligning with the typical time frame for seroconversion. However, variations in sensitivity were observed, influenced by factors such as age, disease severity, and the specific antibody isotypes being detected [12,13].

The role of T cell responses in COVID-19immunity highlighted the complexity of the immune response, showing the need for surveillance strategies encompassing both humoral and cellular immunity [14,15].

Despite the advancements in diagnostic technologies, the dynamic nature of SARS-CoV-2 and the evolving pandemic necessitated continuous evaluation of diagnostic tools. Therefore, this research paper aimed to compare two distinct serological assays for the detection of antibodiegainst SARS-CoV-2 in individuals who had received vaccinations.

METHODOLOGY

This cross-sectional study was conducted at the Section of Chemical Pathology, Department of Pathology, DIMC, from August 1, 2023, to January 31, 2024. A non-probability convenience sampling technique was utilized to select 187 eligible participants, consisting of laboratory personnel aged 18 years and above. Exclusions were pregnant women, individuals with autoimmune diseases, and non-consenting participants. Based on the findings from Mahmoud et al.,[16]. The Roche Elecsys Anti-SARS-CoV-2-

Cobas assay demonstrated a sensitivity of 100% and a specificity of 41.67%. Using a precision of 0.10 and a confidence level of 95%, we calculated the required sample size of 187 participants using an online sample size calculator (https://wnarifin.github.io/ssc/sssnsp.html). Ethical approval was obtained from the Institutional Review Board of DUHS, and all participants provided written informed consent. Blood samples, totaling 5 mL per participant, were collected under aseptic conditions, processed to obtain serum, and stored at -20°C until analysis.

The Siemens Healthineers Atellica®IM SARS-CoV-2 IgG (sCOVG) assay, conducted using the Atellica IM Analyzer, employed a chemiluminescent sandwich immunoassay format to detect IgG antibodies against SARS-CoV-2. Serum samples and controls were incubated with immobilized SARS-CoV-2 antigens, followed by washing and chemiluminescent substrate addition to measure emitted light, providing qualitative (positive/negative) and semi-quantitative (AU/mL) results.

Statistical analyses were conducted using SPSS version 26.0 and R programming. Descriptive statistics, including mean and standard deviation, were computed. The Wilcoxon signed-rank test was employed for paired antibody concentrations. Bivariate analyses utilized scatter plots with regression lines and Pearson correlation coefficients to examine relationships between variables. Additionally, Bland-Altman plots were used to assess agreement between assays, and Passing-Bablok regression was performed. All analyses were conducted at a significance level of $p < 0.05$.

RESULTS

The study included a total of 187 vaccinated individuals, comprising 72 (38.5%) males and 115 (61.5%) females. Regarding SARS CoV-2 antibodies status, 70 participants (37.4%) tested positive, while 117 (62.6%) tested negative with both assays. Analysis of antibody reactivity showed that 91 participants (48.7%) tested reactive for Siemens assay, while 96 (51.3%) were non-reactive. Similarly, for Roche assay, 111 participants (59.4%) showed reactive results and 76 (40.6%) were non-reactive (Table 1).

In the bivariate regression analyses (Fig 2), age showed a weak negative correlation with Siemens, but had minimal explanatory power (near-zero R2). Gender and Covid-19 vaccination status had negligible effects, with coefficients near zero and low R2 values.

In the bivariate regression analyses (Fig 3), age, gender, Covid-19 status, and vaccination status showed weak or no correlation with Roche. Scatter plots and regression lines revealed no clear trends, with near-zero R2 values indicating minimal explanatory power. Thus, these factors did not influence Roche.

The Bland-Altman plot (Fig 4) compares Siemens and Roche assays by showing mean measurements on the x-axis and differences on the y-axis. The red line at 0 suggests minimal bias between assays, with most data points within the limits of agreement (green and cyan lines),indicating good concordance. A few outliers at the extremes suggest areas for further investigation. Overall, both assays reliably detect SARS-CoV-2 antibodies.

Table 1: SARS Cov-2 Antibodies Status with both assays

Table 1	Frequency	Present
Gender		
Male	72	38.5
Female	115	61.5
Positive	70	37.4
Negative	117	62.6
Siemens Reactivity		
Reactive	91	48.7
Non-Reactive	96	51.3
Roche Reactivity		
Reactive	111	59.4
Non-Reactive	76	40.6

The Passing Bablok regression analysis (Fig 5) reveals a proportional relationship between Siemens and Roche assays, with Roche values 0.04 higher than Siemens values, and no intercept bias at zero concentration. However, the Pearson's coefficient of determination (r = 0.297) indicates moderate correlation, reflecting notable variability around the fitted line despite the overall positive trend observed between the two assays.

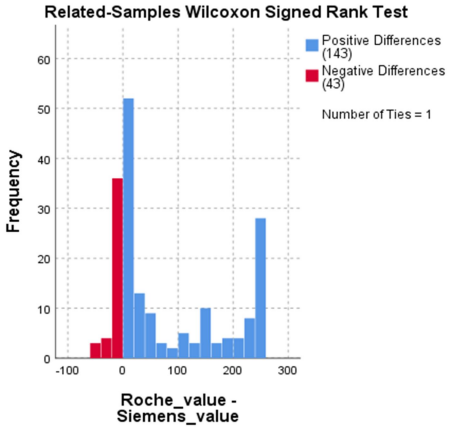
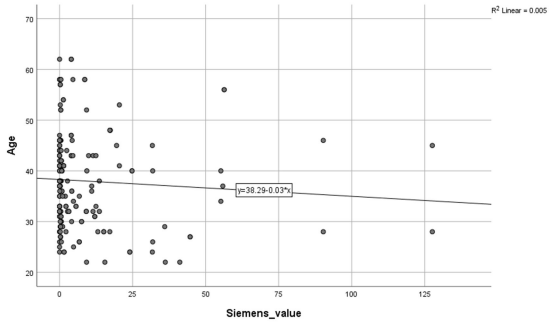
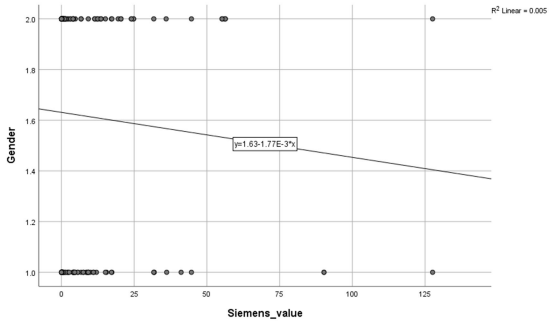
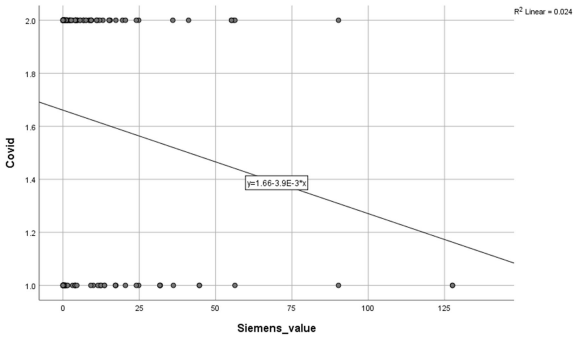


Figure 1: Comparison of Roche and Siemens with Fitted Regression Line







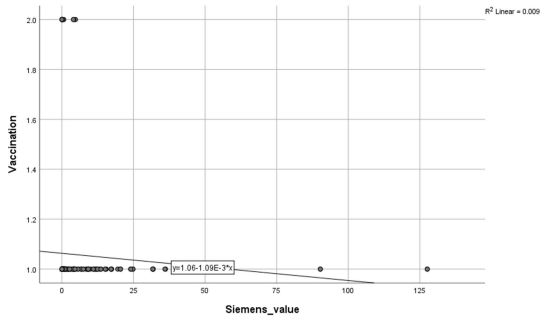


Figure 2: Factors Influencing Siemens in Bivariate Regression Analyses: a) Age, b) Gender, c)Covid-19 Status and d)Vaccination Status

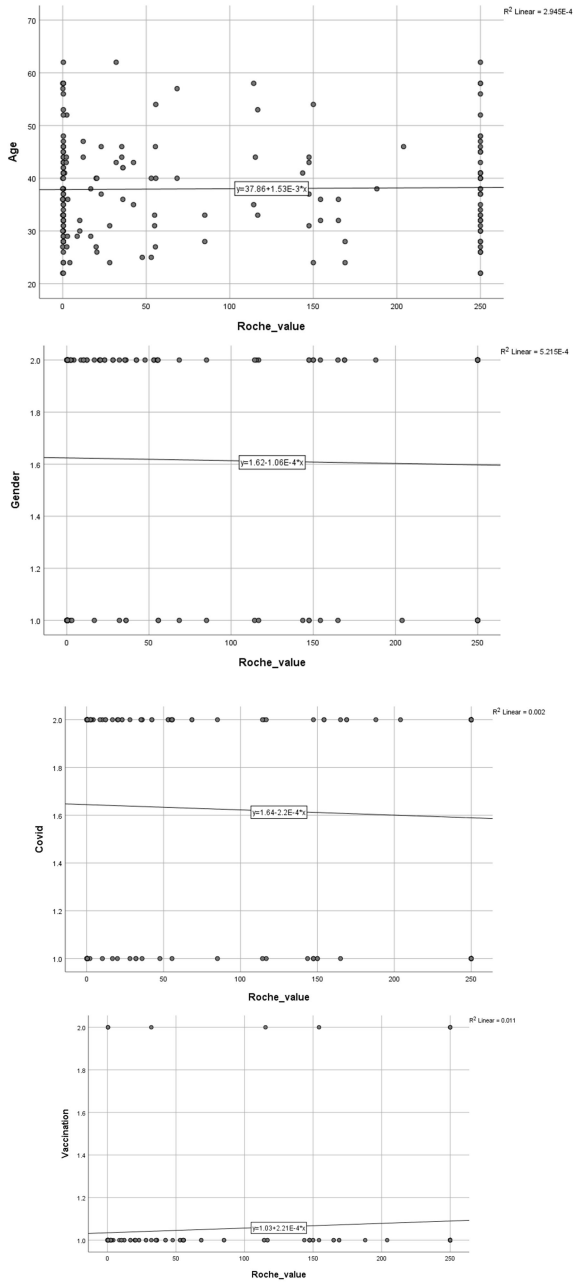


Figure 3: Factors Influencing Roche Value in Bivariate Regression Analyses: a) Age, b) Gender, c) Covid-19 Status and d) Vaccination Status

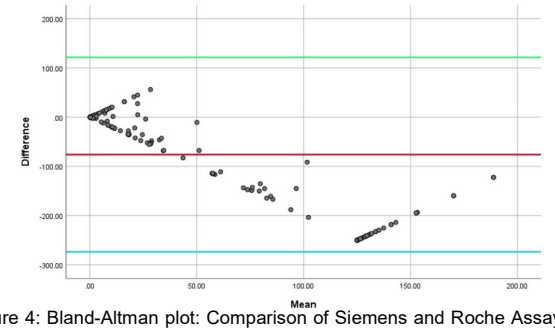


Figure 4: Bland-Altman plot: Comparison of Siemens and Roche Assays for Antibody Detection against SARS-CoV-2

Passing Bablok Regression Fit

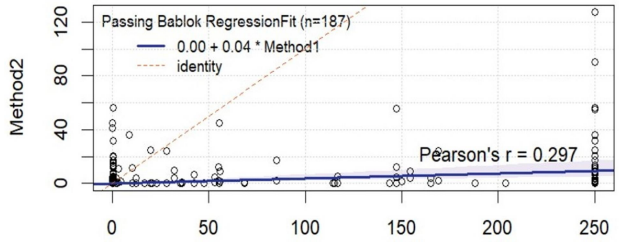


Figure 5: Passing Bablok Regression Analysis: Comparison of Siemens and Roche Assays for Antibody Detection against SARS-CoV-2

DISCUSSION

The COVID-19-19 pandemic has significantly impacted global health systems and research priorities. Accurate diagnostic tools, particularly serological assays, are crucial for understanding the pandemic and evaluating immune responses after vaccination. This study compared Siemens Healthineers Atellica® IM SARS-CoV-2 IgG and ROCHE Elecsys® Anti-SARS-CoV-2 for detecting antibodies against SARS-CoV-2 in individuals who have received vaccinations.

Our study found that Roche assays generally yielded higher antibody levels compared to Siemens. This observation is consistent with studies such as Müller et al. [17], Perkmann et al. [18], Giavarina et al. [19] reported Roche assays as having higher values compared to other assays. Conversely, Siemens assay, although reliable, exhibited slightly lower reactivity compared to Roche, as observed in studies by Kim et al. [20] and Irsara et al. [21]. Roche's double-antigen sandwich format and electrochemiluminescence detection likely enhance its performance, as supported by Chan et al., [22]. Initially, Roche assays showed lower antibody levels post-vaccination but significantly higher (5-6 times) compared to other assays [23]. This advantage is due to Roche's method, which maintains stable or increasing antibody levels over time, unlike assays with secondary antibodies that decline [24]. Additionally, Roche's assay measures antibody affinity, resulting in higher values for mature, high-affinity antibodies [25]. Consequently, Roch demonstrated elevated antibody levels in sera collected months after infection or vaccination.

Our bivariate regression analyses showed weak correlations between demographic factors (age, gender) and antibody levels. The Bland-Altman plot indicated good concordance between Siemens and Roche assays, though some outliers were present. This is consistent with studies by Jeong et al. [26], which reported overall agreement between different serological tests while noting variability due to assay-specific factors. The presence of outliers in our study highlights the need for further investigation into the causes of these discrepancies, such as differences in assay calibration or sample handling. Our Passing-Bablok regression analysis showed a moderate correlation with Roche values generally higher than Siemens values, corroborated by study such

as Jeong et al. [26]. These findings emphasize the importance of considering assay-specific characteristics when interpreting serological data, as differences in assay methodologies can lead to varying results.

This study has few limitations. The sample size of 187 laboratory personnel may not fully represent the general population, affecting the generalizability of the results. The cross-sectional design limits our ability to track changes in antibody levels over time or assess long-term assay performance. We did not measure antibody affinity directly, which could impact the interpretation of Roche's higher values. Convenience sampling may introduce bias, and the lack of clinical correlation with patient outcomes.

CONCLUSION

Our study demonstrated that the ROCHE Elecsys® Anti-SARS-CoV-2 assay generally provided higher antibody levels compared to the Siemens Healthiness Atellica® IM SARS-CoV-2 IgG assay in vaccinated individuals. These findings highlight the importance of considering assay-specific characteristics when interpreting serological data. Future studies with larger and more diverse populations are recommended to further validate these results and explore the implications for ongoing public health efforts.

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Conflict of Interest: None

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