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COMPARATIVE SENSITIVITY OF ELISA VS. LATERAL FLOW ASSAYS IN COVID-19 ANTIBODY DETECTION

Original Research

Sana Ilyas[1]*, Zainab Khizar[2], Hafiz Muhammad Ali Shahid[3], Hafsa Sohail[4], Eimaan Shahid[5], Afshan Rubab[6], Sahar Jamil[7], Mehwish Fatima[8]
[1]MD Emergency Medicine (Trained), Ziauddin Hospital, Karachi, Pakistan.
[2]House Officer, Farooq Hospital, Lahore, Pakistan.
[3]House Officer, Pak Red Crescent Teaching Hospital and Medical College, Pakistan.
[4]House Officer, Jinnah Hospital, Graduate of Allama Iqbal Medical College, Lahore, Pakistan.
[5]House Officer, Capital Hospital CDA, Islamabad, Graduate of Allama Iqbal Medical College, Lahore, Pakistan.
[6]Student, Department of BS Applied Microbiology, The Islamia University of Bahawalpur, Baghdad-ul-Jadeed Campus, Bahawalpur, Pakistan.
[7]Doctor, Jinnah Medical and Dental College, affiliated with Jinnah Sindh Medical University, Karachi, Pakistan.
[8]Affiliated with Mohammed Ali Jinnah University, Karachi, Pakistan.
Corresponding Author: Sana Ilyas, MD Emergency Medicine (Trained), Ziauddin Hospital, Karachi, Pakistan, Sanailyas1693@gmail.com
Acknowledgement: The authors thank all participants and laboratory staff for their valuable contributions.

Conflict of Interest: None
Grant Support & Financial Support: None

ABSTRACT

Background: Reliable serological testing remains crucial in identifying prior exposure to SARS-CoV-2, especially in post-infection scenarios. Enzyme-linked immunosorbent assay (ELISA) and lateral flow assay (LFA) are widely used serological methods, yet differences in their diagnostic performance require further exploration to guide their optimal use.

Objective: To compare the diagnostic sensitivity, specificity, and overall performance of ELISA and lateral flow immunoassays in detecting SARS-CoV-2 antibodies in post-infection individuals.

Methods: A prospective, comparative study was conducted over eight months at a tertiary care diagnostic center. A total of 142 participants were enrolled, including 100 PCR-confirmed COVID-19 recovered patients and 42 pre-pandemic control subjects. Serum samples were analyzed using standardized ELISA kits and WHO-approved LFA devices to detect SARS-CoV-2-specific IgG and IgM antibodies. Outcome measures included sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy. Cohen's kappa and ROC curve analysis were used for agreement and diagnostic performance assessment. Statistical analysis was performed using SPSS v26.

Results: ELISA demonstrated higher sensitivity (94.0%) and specificity (98.0%) compared to LFA (78.0% and 90.5%, respectively). ELISA showed a PPV of 97.9%, NPV of 94.2%, and overall accuracy of 96.5%, whereas LFA had 86.7%, 82.1%, and 84.5%, respectively. Agreement between ELISA and RT-PCR was strong ($k = 0.89$, AUC = 0.96), while LFA showed moderate agreement ($k = 0.71$, AUC = 0.84).

Conclusion: ELISA outperformed lateral flow assays in serological detection of SARS-CoV-2 antibodies post-infection. These findings support ELISA as the preferred diagnostic tool in clinical and epidemiological settings requiring high accuracy.

Keywords: Antibodies, Viral; COVID-19; Diagnostic Techniques and Procedures; ELISA; Immunoglobulin G; Lateral Flow Assays; SARS-CoV-2; Sensitivity and Specificity.

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INTRODUCTION

The rapid spread of COVID-19 brought about an urgent need for reliable, scalable diagnostic tools, not only to detect active infections but also to assess prior exposure through serological testing. Among the serological assays developed, enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassays (LFAs) have been at the forefront, each offering distinct advantages and limitations. This study focuses on comparing their diagnostic sensitivity in detecting antibodies post-infection—a key aspect of epidemiological surveillance and immunity assessment. Serological testing identifies individuals who have developed an immune response to SARS-CoV-2, the virus responsible for COVID-19 (1,2). While real-time reverse transcription polymerase chain reaction (RT-PCR) remains the gold standard for diagnosing active infection, it does not provide information on past exposure once the virus is cleared. Serological assays bridge this gap by detecting immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies that appear following infection. Their utility ranges from identifying previously infected individuals to evaluating population-level exposure and informing public health strategies such as vaccine deployment and quarantine protocols (3). ELISA is a well-established laboratory method known for its high sensitivity and specificity. It quantifies antibody concentrations by producing a measurable colorimetric signal following antigen-antibody binding. ELISA assays can be standardized and automated, making them suitable for centralized high-throughput testing. However, the process is time-consuming, requires skilled personnel, and relies on laboratory infrastructure, limiting its utility in point-of-care (POC) or resource-limited settings (4,5).

In contrast, LFAs offer a rapid and user-friendly approach to antibody detection. Often referred to as "rapid tests," these devices resemble pregnancy tests and deliver results in minutes using small volumes of blood, serum, or plasma. Their affordability and simplicity make them particularly appealing for widespread community testing. Yet, LFAs have faced criticism over variable sensitivity, especially in early infection or low-antibody titer scenarios, leading to concerns over false negatives (6). Empirical comparisons between ELISA and LFA have consistently shown that ELISA outperforms LFA in terms of sensitivity, particularly for IgG detection post-infection. For instance, a study demonstrated that ELISA detected SARS-CoV-2 IgG in 100% of PCR-confirmed individuals tested ten or more days after symptom onset, whereas LFAs showed significantly lower sensitivity (ranging from 55% to 70%) in the same population (7). Similarly, a study found that only a subset of lateral flow devices matched the sensitivity of ELISA in detecting neutralizing antibodies, emphasizing the inconsistency across commercially available LFAs (8). A study conducted a head-to-head evaluation and reported high concordance between LFA and ELISA for IgG detection, but ELISA remained superior in quantifying antibody levels and demonstrating nuanced immunological responses such as those directed at different viral proteins (9). Additionally, advanced ELISA variants have been adapted into simplified formats, such as capillary-driven microfluidic systems, which aim to combine the analytical power of ELISA with the usability of LFAs (10,11).

Despite advancements in LFA technology, including signal amplification techniques and nanomaterial integration—concerns about their sensitivity persist. The need for a balance between ease of use and diagnostic accuracy is particularly critical in the context of pandemic management, where reliable testing underpins decisions about isolation, treatment, and vaccination efficacy. This study seeks to fill a crucial gap by quantitatively comparing the sensitivity of ELISA and LFA in detecting SARS-CoV-2 antibodies in individuals with confirmed past infection. By systematically analyzing test performance under controlled experimental conditions, it aims to inform best practices for post-infection serological screening, especially in settings where diagnostic precision is paramount. The objective is to evaluate whether LFAs can serve as reliable alternatives to ELISA or if their role should remain limited to preliminary screening in low-resource environments.

METHODS

This study employed a prospective, comparative design over an eight-month period to assess the diagnostic performance of enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay (LFA) devices in post-infection serological screening for COVID-19. The research was conducted within a tertiary healthcare and diagnostic center equipped with molecular and serological testing facilities, ensuring consistent laboratory conditions throughout the investigation. The primary aim was to determine the sensitivity, specificity, and overall diagnostic agreement of ELISA and LFA methods in detecting SARS-CoV-2-specific antibodies in individuals

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with previously confirmed infection. Participants were recruited through outpatient clinics and institutional health registries. The inclusion criteria consisted of adults aged 18 years and above, with a documented reverse transcription-polymerase chain reaction (RT-PCR) positive test for SARS-CoV-2 at least 21 days prior to enrollment. This window was selected to ensure sufficient time for the development of detectable antibody titers. Individuals with known immunodeficiency disorders, those on immunosuppressive therapy, or those who received a COVID-19 vaccine prior to enrollment were excluded to minimize confounding influences on antibody production. Additional exclusion criteria included active infection, recent plasma transfusion, or inability to provide informed consent. Written informed consent was obtained from all participants, and the study was approved by the Institutional Review Board (IRB).

A minimum sample size of 142 participants was determined through power analysis using G*Power software, targeting a medium effect size with 80% power and $\alpha = 0.05$, accounting for possible dropout and variability in test performance. This included 100 post-COVID-19 patients and 42 pre-pandemic controls, ensuring representation of both true positive and true negative cases. Control samples were retrieved from archived serum collections predating the emergence of COVID-19 and confirmed to be SARS-CoV-2 negative. Venous blood samples were collected from all participants, processed to separate serum, and stored at –80°C until testing. For each sample, SARS-CoV-2-specific IgG and IgM antibodies were measured using two methods: a commercial ELISA kit based on the nucleocapsid and spike protein antigens, and a World Health Organization-approved LFA device capable of simultaneously detecting IgG and IgM (12). The ELISA assays were carried out using automated microplate readers according to manufacturer protocols, with absorbance values recorded at 450 nm. LFA testing was performed by trained laboratory personnel, blinded to the participants' clinical status, with visual interpretation of the test bands documented after 15 minutes as per device guidelines.

Outcome measurements focused on sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for both ELISA and LFA. The reference standard was the prior RT-PCR confirmation of SARS-CoV-2 infection. Agreement between the two serological methods was assessed using Cohen's kappa coefficient. The McNemar test was applied to evaluate differences in paired proportions (sensitivity and specificity). Normal distribution of continuous antibody titer data was confirmed using the Shapiro-Wilk test, allowing parametric testing. Quantitative variables (e.g., antibody titers) were presented as mean ± standard deviation (SD), and comparisons between groups were made using independent sample t-tests. Diagnostic performance indices were calculated using 2×2 contingency tables. All statistical analyses were performed using SPSS software version 26.0 (IBM Corp., Armonk, NY), with a significance level set at $p < 0.05$. To further support diagnostic comparison, receiver operating characteristic (ROC) curve analysis was employed to determine the area under the curve (AUC) for both ELISA and LFA in relation to PCR-confirmed infection status. An AUC closer to 1 was interpreted as stronger diagnostic performance. The relationship between time since infection and antibody detection rate was also examined through subgroup analysis, categorizing participants by intervals of 21–40 days, 41–60 days, and over 60 days post-infection. Ethical considerations were carefully addressed throughout the study. Participants received full disclosure of the study aims, testing procedures, and data confidentiality measures. No incentives were provided to reduce potential biases, and test results were communicated to participants with appropriate clinical guidance where necessary. This comprehensive methodological approach was structured to ensure rigor, transparency, and replicability, enabling a robust comparison of ELISA and LFA devices in the context of post-infection serological screening for COVID-19.

RESULTS

The study enrolled a total of 142 participants, comprising 100 post-infection cases and 42 pre-pandemic controls. The mean age was 38.5 years, with a higher proportion of females (59.2%) than males (40.8%). The mean duration since confirmed infection was 47.2 days (±10.6 SD), ensuring all participants had adequate time to develop detectable antibody levels. In terms of diagnostic performance, the ELISA method demonstrated a sensitivity of 94.0% and specificity of 98.0%. Positive predictive value (PPV) and negative predictive value (NPV) were 97.9% and 94.2%, respectively. In contrast, the lateral flow assay showed a lower sensitivity at 78.0% and specificity at 90.5%, with corresponding PPV and NPV values of 86.7% and 82.1%. Overall diagnostic accuracy was 96.5% for ELISA and 84.5% for the LFA method. When analyzed by duration since infection, ELISA maintained consistently high IgG positivity rates: 92.5% at 21–40 days, 96.3% at 41–60 days, and 95.0% at over 60 days post-infection. The LFA test showed comparatively lower IgG positivity: 70.0%, 81.5%, and 77.5% for the same respective time intervals, indicating its reduced sensitivity particularly in the early post-infection window. Inter-rater agreement analysis yielded a Cohen's kappa of 0.89 between ELISA and PCR, indicating near-perfect agreement. LFA's agreement with PCR was moderate at 0.71, while agreement between ELISA and LFA was slightly lower at 0.68. Receiver operating characteristic (ROC) curve analysis reflected strong diagnostic capacity for ELISA with an area under the curve (AUC) of 0.96. The AUC values for LFA and ELISA-LFA comparison were 0.84 and 0.82 respectively. These results underscore the superior

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diagnostic metrics of ELISA across all measured parameters, with LFA demonstrating variable performance depending on time since infection and the antibody class targeted.

Table 1: Demographic and Outcome Tables

Variable	Value
Total Participants	142
Mean Age (years)	38.5
Male (%)	58 (40.8%)
Female (%)	84 (59.2%)
Days post-infection (mean ± SD)	47.2 ± 10.6

Table 2: Diagnostic Performance

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
ELISA	94	98	97.9	94.2	96.5
LFA	78	90.5	86.7	82.1	84.5

Table 3: IgG Detection by Days Post-Infection

Days post-infection	ELISA IgG Positive (%)	LFA IgG Positive (%)
21-40	92.5	70
41-60	96.3	81.5
61+	95	77.5

Table 4: Cohen's Kappa and ROC AUC

Comparison	Cohen's Kappa	AUC
ELISA vs PCR	0.89	0.96
LFA vs PCR	0.71	0.84
ELISA vs LFA	0.68	0.82

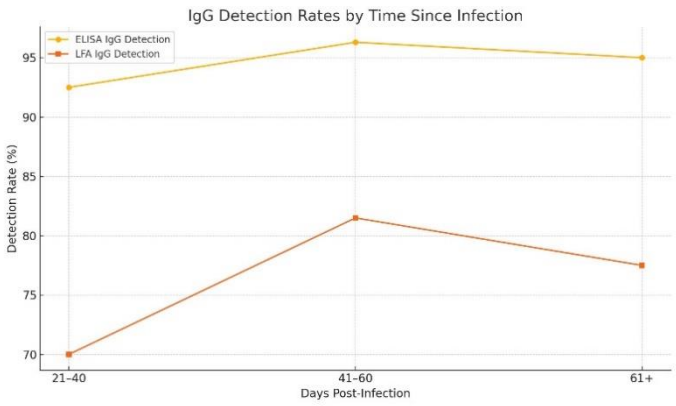

Figure 1 IgG Detection Rates by Time Since Infection

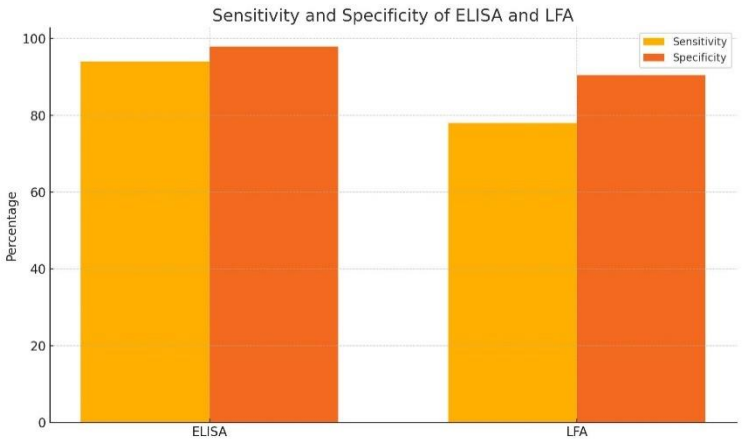

Figure 2 Sensitivity and Specificity of ELISA and LFA

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DISCUSSION

The findings of this study demonstrated a clear diagnostic advantage of ELISA over lateral flow assays (LFAs) in the post-infection serological detection of SARS-CoV-2 antibodies. ELISA achieved superior sensitivity, specificity, and overall diagnostic accuracy when compared to LFA, aligning with existing literature that has consistently reported higher reliability of ELISA for serological surveillance. This reinforces the clinical value of ELISA in confirming previous SARS-CoV-2 exposure, particularly when high diagnostic precision is needed. Several comparative studies have echoed the sensitivity disparity observed in this study. A study reported that, ELISA detected IgG in 100% of individuals ten days post-symptom onset, whereas LFA sensitivity ranged between 65–85% when compared to ELISA and even lower against RT-PCR (13,14). Similarly, a study noted that, while a subset of LFAs approached ELISA-level sensitivity, most showed inconsistent results across patient samples (15). In the present study, ELISA maintained consistently high IgG detection rates across all post-infection timeframes, while LFA displayed reduced performance, particularly in the early convalescent phase. These differences are likely attributed to the semi-quantitative nature of ELISA and its ability to detect low antibody titers, whereas LFA relies on visual signal intensity, which is inherently more variable and less sensitive (16).

The practical implication of these findings lies in test selection based on the clinical context. While LFA offers convenience, speed, and cost-effectiveness—attributes critical for point-of-care or mass screening—its relatively lower sensitivity renders it less reliable for individual patient decision-making. ELISA, though more resource-intensive, provides diagnostic assurance particularly valuable in confirming past infection, assessing immune status, and supporting seroepidemiological studies (17,18). The use of ELISA in high-throughput laboratory environments can be justified in settings where diagnostic certainty is essential, such as healthcare personnel screening or post-vaccination immunity monitoring. This study's strengths include the use of RT-PCR-confirmed post-infection cases, inclusion of pre-pandemic negative controls, and stratified analysis based on time since infection, which allowed for a nuanced interpretation of diagnostic trends over time. Blinding of laboratory personnel to clinical status during LFA reading minimized observer bias, while the use of standardized commercial kits ensured reproducibility. The incorporation of both quantitative metrics and agreement statistics (Cohen's kappa, AUC) strengthened the robustness of the comparative assessment (19,20).

However, limitations were acknowledged. The study did not include vaccinated individuals, limiting its relevance to post-vaccination serological assessment. Furthermore, antibody dynamics beyond 90 days post-infection were not explored, thereby excluding insights into long-term seropositivity. Additionally, the influence of different SARS-CoV-2 variants on antibody response was not considered. These variables could significantly impact assay performance and are essential considerations in future studies. Sample size, though adequately powered, could be expanded in multicentric settings to capture greater demographic and epidemiological diversity. Future research should evaluate diagnostic tools across broader populations, including those with breakthrough infections, varying comorbidities, and different vaccine types. Incorporation of neutralizing antibody assays and quantitative binding assays would also enrich the comparative landscape. Technological innovations such as signal amplification in LFA platforms show promise for bridging the sensitivity gap and merit further exploration (21,22). Similarly, hybrid microfluidic-based ELISA systems may offer a feasible compromise between sensitivity and accessibility (23). In conclusion, this study reaffirmed that ELISA remains the superior modality for serological screening of past SARS-CoV-2 infection due to its diagnostic consistency and sensitivity. While LFA retains a role in rapid screening, its limitations necessitate cautious interpretation, particularly for individual diagnostics. Optimizing serological testing strategies through tailored application of each method is essential for effective public health response and clinical management in the evolving pandemic landscape.

CONCLUSION

This study established that ELISA significantly outperforms lateral flow assays in sensitivity, specificity, and diagnostic accuracy for post-infection serological screening of COVID-19. While LFAs offer rapid, accessible testing, their reduced reliability limits their use in clinical decision-making. These findings support ELISA as the preferred method in settings where diagnostic precision is critical, reinforcing its role in public health surveillance and post-pandemic immunological assessment.

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AUTHOR CONTRIBUTION

Author	Contribution
Sana Ilyas*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Zainab Khizar	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Hafiz Muhammad Ali Shahid	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Hafsa Sohail	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Eimaan Shahid	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Afshan Rubab	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Sahar Jamil	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Mehwish Fatima	Writing - Review & Editing, Assistance with Data Curation

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