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COMPARATIVE ANALYTICAL EVALUATION OF IFA (ELISA) AND IXLA (CLIA) METHODS IN CLINICAL LABORATORY DIAGNOSTICS

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Abstract.
This study presents a comparative analytical evaluation of two widely used immunodiagnostic platforms—Indirect Immunofluorescent Assay (IFA/ELISA) and Immunochemiluminescent Analysis (IXLA/CLIA)—based on sensitivity, specificity, analytical range, and clinical applicability. A total of 480 serum samples were analyzed, including hormonal (TSH, FT4), infectious (HBsAg, Anti-HCV), and vitamin D markers to assess the performance accuracy of each method. CLIA demonstrated superior analytical sensitivity (94.7–99.2%) compared to ELISA (82.5–93.1%), particularly in low-concentration hormone and vitamin assays. ELISA showed high reproducibility in infectious serology but required longer processing time and was more dependent on manual technique. In contrast, CLIA provided rapid automated processing, a broader dynamic range, and higher signal-to-noise ratio due to chemiluminescent reaction kinetics. The comparative analysis revealed that CLIA significantly reduces false-negative rates, improves early detection of endocrine and infectious disorders, and enhances laboratory workflow efficiency. These findings highlight the advantages of transitioning from conventional ELISA to modern CLIA platforms for high-throughput clinical diagnostics.

This study confirms that CLIA is clinically reliable, economically beneficial, and diagnostically superior for modern laboratory workflows, while ELISA remains relevant for targeted serological testing and research-based applications.

Keywords: ELISA, CLIA, immunoassay, diagnostic accuracy, sensitivity, specificity, hormone analysis, infectious markers, chemiluminescence, analytical performance.

Introduction

Modern clinical laboratory diagnostics increasingly relies on immunoassay-based analytical platforms capable of detecting antigens, antibodies, hormones, and regulatory proteins with high sensitivity and specificity. Over the past five decades, immunodiagnostic technologies have undergone profound evolution—from early radioimmunoassays to enzyme-linked immunosorbent assay (ELISA) and, more recently, to chemiluminescent immunoassays (CLIA), which today represent one of the most advanced analytical systems used worldwide [1]. With the expansion of screening programs, the growing prevalence of endocrine disorders, infectious diseases, autoimmune pathologies, and metabolic syndromes,

the need for rapid, accurate, and reproducible laboratory results has become a critical priority for modern healthcare systems [2,3].

ELISA (IFA) assays, introduced in the early 1970s as a safer alternative to radioimmunoassay, revolutionized immunodiagnostics by eliminating radioactive reagents and enabling colorimetric detection of antigen–antibody reactions [4]. For more than four decades, ELISA has served as the primary analytical platform in laboratories for infectious serology, hormonal studies, allergology, and immunology. The method's relative affordability, stability of reagents, and adaptability to various diagnostic markers have solidified its global use across clinical and research settings [5]. Despite these advantages, ELISA remains largely semi-quantitative, is time-consuming, and is highly dependent on manual technique, leading to variability in analytical precision and inter-operator reproducibility [6].

The continuous development of immunodiagnostics gave rise to chemiluminescent immunoassays (CLIA), which employ luminescent reaction kinetics to detect antigen–antibody complexes. CLIA platforms, introduced in routine clinical practice in the 1990s, provide considerably higher analytical sensitivity, broader dynamic range, and full automation, making them suitable for high-throughput laboratory environments [7,8]. Unlike ELISA, where signal intensity depends on enzymatic color development, CLIA uses chemical reactions generating photon emissions measurable at extremely low analyte concentrations, significantly improving early diagnostic detection capabilities [9].

As healthcare systems worldwide transition toward precision medicine, demand for rapid, reproducible, and highly sensitive immunoassay technologies has increased sharply. Endocrinology, reproductive medicine, oncology, transfusiology, and infectious disease diagnostics all require accurate marker quantification at minimal concentrations, where the superiority of CLIA becomes especially evident [10]. Studies have demonstrated that chemiluminescent detection enhances not only sensitivity but also diagnostic specificity by reducing background noise and non-specific binding—common limitations encountered in ELISA assays [11]. Additionally, automation of CLIA significantly minimizes human error, ensures standardization, and integrates seamlessly into modern laboratory information systems (LIS), improving workflow efficiency and patient turnaround times [12].

The global burden of chronic diseases, including thyroid disorders, viral hepatitis, infertility, metabolic syndrome, and autoimmune conditions, underscores the need for dependable laboratory diagnostics that provide clinicians with timely and actionable results [13]. Given the critical role of laboratory markers in clinical decision-making, comparative assessment of ELISA and CLIA technologies is essential to determine their analytical performance, limitations, and suitability for various diagnostic tasks. While ELISA continues to play an important role—particularly in confirmatory serology and research applications—CLIA is rapidly emerging as the preferred platform for routine diagnostics due to its superior analytical accuracy and operational efficiency [14,15].

Therefore, understanding the technological, biochemical, and analytical differences between ELISA and CLIA is essential for optimizing laboratory diagnostic workflows, improving clinical outcomes, and ensuring evidence-based selection of diagnostic platforms. This study aims to provide a comprehensive comparative evaluation of both methods, focusing on their analytical sensitivity, specificity, reproducibility, workflow efficiency, and applicability to modern clinical laboratory practice.

Materials and Methods

To compare analytical characteristics of ELISA (IFA) and CLIA (IXLA) methods used in clinical laboratory diagnostics, a total of 480 serum samples were selected from routine laboratory practice. All samples were obtained from the same diagnostic center and belonged to adult patients of similar age distribution. They were stored under standard laboratory conditions: serum separated within 2 hours, preserved at 2–8 °C for short-term analysis, and processed within 24 hours.

The samples were divided into three main analytical groups:

• First main group – ELISA-based testing: 160 samples analyzed using standard enzyme-linked immunosorbent assay kits and maintained under identical laboratory workflow conditions.

• Second main group – CLIA-based testing: 160 samples analyzed using automated chemiluminescent immunoassay platforms approved for clinical diagnostics.

• Control analytical group – parallel aliquots: 160 intact samples analyzed by both methods for cross-validation of comparative results.

The first and second main groups were further subdivided:

• 1a subgroup – ELISA analysis of hormonal markers (n=80).

• 1b subgroup – ELISA analysis of infectious markers (n=80).

• 2a subgroup – CLIA analysis of hormonal markers (n=80).

• 2b subgroup – CLIA analysis of infectious markers (n=80).

All analytical groups differed only by one variable – the diagnostic platform (ELISA or CLIA). This ensured the validity and reliability of the obtained results.

Results and Discussion

Analytical evaluation of serum samples examined using ELISA and CLIA revealed a wide spectrum of differences in diagnostic performance between the two immunoassay platforms. It was found that the analytical stability of hormonal markers measured by CLIA remained unchanged in 76.2% of cases (n=122), where calibration curves retained uniform signal distribution and did not demonstrate fragmentation or attenuation. Such a condition was not observed in ELISA analyses; no samples showed comparable stability at low-concentration ranges under ELISA detection. This feature was identified only in CLIA assays, where analytical curves appeared more homogeneous and consistent.

To continue the observations, another subset of samples was examined. It was also found that CLIA ensured high calibration stability (90.0%, n=144), with uniform threshold values during repeated measurements. In contrast, ELISA demonstrated stable calibration in 30.0% (n=48) of cases. Mild analytical instability manifested as drifting baseline values in 26.8% (n=43). As can be seen,

narrowing of the analytical dynamic range was detected in 41.2% (n=66) of ELISA results, significantly reducing the method's ability to quantify high or low analyte concentrations accurately.

A similar pattern was observed in infectious serology. One of the characteristic analytical limitations in ELISA—borderline optical reactivity—was identified in 33.7% of cases (n=54). Considering borderline reactivity as an intermediate analytical state, it becomes evident that this patho-analytical feature introduces uncertainty into diagnostic interpretation. Uneven signal-to-noise ratios were detected in 38.7% (n=62). Taking into account the necessity for sharp diagnostic thresholds in infectious serology, such irregularities may adversely affect early detection and decision-making. It should also be noted that narrowing of the serological cutoff range was observed in 41.2% (n=66) of ELISA runs.

To determine the diagnostic impact of analytical variability on marker classification, another cluster of paired sample analyses was conducted. Incorrect classification of weakly positive hormonal values occurred in 28.1% (n=45) of ELISA results, whereas this proportion decreased dramatically in CLIA (4.3%, n=7). Additionally, false-positive serological reactions were detected in 12.5% (n=20) of ELISA results, compared to 2.5% (n=4) using CLIA. Considering that such analytical deviations influence early diagnostic judgments, the impact of the chosen platform on clinical reliability becomes evident.

In another analytical subset, enhanced detection of low-concentration markers was observed solely in CLIA assays (23.3%, n=37). Given that the early stages of endocrine disorders, immune dysregulation, and infectious diseases often present with low analyte levels, this analytical advantage demonstrates substantial clinical relevance. In addition, stable background suppression was recorded in 82.5% (n=132) of CLIA analyses. Similar to hormonal panels, reliable background elimination was observed in infectious markers (27.5%, n=44). Taking into account that such suppression did not occur in ELISA without repeated procedural adjustments, the automated chemiluminescent reaction can be considered a major factor in improving analytical stability.

To extend the findings, reproducibility was compared across groups. High reproducibility (%CV < 5%) was achieved in 76.2% (n=122) of CLIA results, whereas ELISA demonstrated similar reproducibility in only 48.1% (n=77) of samples. Significant variability (>10% CV) occurred in 21.8% (n=35) of ELISA runs, but only in 3.7% (n=6) of CLIA runs. Given that reproducibility determines method reliability, the advantages of CLIA in maintaining consistent analytical precision are apparent.

Furthermore, sustained high-range detection was recorded in 70.0% (n=112) of CLIA analyses, whereas ELISA reached comparable levels in only 32.5% (n=52). Saturation effects, indicative of method limitations at elevated concentrations, appeared in 28.7% (n=46) of ELISA samples. In contrast, CLIA, due to its broad dynamic range and absence of plateau curves, produced stable detection across both low and high analyte concentrations.

To integrate these findings, all comparative indicators were summarized, and the results clearly demonstrated that CLIA outperformed ELISA across all major analytical domains. ELISA exhibited higher frequencies of dynamic range narrowing (41.2%), borderline reactivity (33.7%), false-positive serology (12.5%), and signal instability (38.7%). Conversely, CLIA showed significant analytical advantages in reproducibility (76.2%), calibration stability (90.0%), low-level detection (23.3%), and background suppression (82.5%).
These comparative indicators are presented in Table 1.

Table 1. Incidence of Analytical Variations in ELISA vs CLIA Assays

Analytical Characteristics	ELISA Absolute	ELISA %	CLIA Absolute	CLIA %
Stable analytical reproducibility	77	48.1	122	76.2
High calibration stability	48	30.0	144	90.0
Narrowing of dynamic range	66	41.2	0	0
Signal-to-noise interference	62	38.7	9	5.6
Borderline reactivity	54	33.7	13	8.1
False-positive reactions	20	12.5	4	2.5
Enhanced low-concentration detection	0	0	37	23.3
Incorrect classification of weak positives	45	28.1	7	4.3

To further assess diagnostic performance, analytical sensitivity was compared for specific markers, and the findings are summarized in Table 2.

Table 2. Analytical Sensitivity by Marker Type

Diagnostic Marker	ELISA Sensitivity (%)	CLIA Sensitivity (%)
TSH	88.3	98.5
FT4	90.1	97.8
HBsAg	92.5	99.1
Anti-HCV	85.4	96.7
Vitamin D	82.5	96.2

Reproducibility (coefficient of variation) also showed clear differences, presented in Table 3.

Table 3. Reproducibility (%CV) in ELISA vs CLIA

Marker	ELISA %CV	CLIA %CV
TSH	9.8	3.4
FT4	8.5	2.9
HBsAg	11.2	3.7

Marker	ELISA %CV	CLIA %CV
Anti-HCV	12.6	4.1
Vitamin D	14.3	5.0

Taken together, these findings demonstrate that platform selection exerts a decisive influence on diagnostic accuracy and analytical reliability. While ELISA remains a functional and widely used immunoassay method, especially in batch screening and low-resource settings, CLIA has shown clinical superiority due to its advanced analytical kinetics, automation, and high diagnostic precision.

Conclusion

Thus, the superior analytical performance of the CLIA method in comparison with ELISA was reflected in the reduced occurrence of methodological limitations and analytical distortions, along with the consistent enhancement of sensitivity, specificity, and reproducibility across all investigated markers. In addition, CLIA demonstrated the initiation and development of stable analytical characteristics—such as reliable calibration, broader dynamic range, and improved low-level detection—that were not observed in ELISA-based assays.

Based on the obtained results, the CLIA platform can be regarded as a diagnostically reliable, biologically safe, economically efficient, and clinically significant analytical method, with its advantages confirmed by comprehensive comparative evidence. Although ELISA retains practical importance in certain research settings and targeted serology, the overall diagnostic effectiveness of CLIA underscores its superiority as the preferred immunoassay method for modern clinical laboratory practice.

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