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THE CHEMILUMINESCENCE METHOD IN MICROBIOLOGY WITH A FOCUS ON SCREENING AND DIAGNOSTIC POSSIBILITIES FOR INFECTIOUS DISEASES

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Abstract. *Chemiluminescent immunoassay (CLIA) is a highly sensitive and specific method used in microbiological laboratories for diagnosing infectious diseases. CLIA utilizes chemiluminescent substrates and enzymes, such as horseradish peroxidase (HRP) and alkaline phosphatase (AP), for detecting antigens or antibodies through a light signal. Several CLIA test formats exist, including direct chemiluminescent immunoassay (CLIA), chemiluminescent enzyme immunoassay (CLEIA), and electrochemiluminescent immunoassay (ECLIA), with each being applied depending on the type of analyte and testing requirements. Modern devices based on the CLIA method offer precise and reliable detection of numerous infectious agents, including viruses, bacteria, and parasites. These devices are equipped with systems for automatic detection of pipetting and sample testing errors, ensuring continuous operation and high efficiency. Application of the CLIA method in microbiology is essential for accurate diagnosis and monitoring of infectious diseases. CLIA is widely used for diagnosing a broad range of infectious diseases caused by microorganisms, including viruses such as HIV, bacteria, and parasites. This method allows for highly accurate identification of pathogens, which is crucial for fast and precise diagnosis and appropriate treatment.*

Key words*: chemiluminescence, immunoassay, microbiology, screening, enzymes*

Introduction

Immunological tests are fundamental tools in modern medical diagnostics, based on the highly specific binding reaction between antigens and antibodies. The technology of chemiluminescent immunoassay (CLIA) emerged in the early 1980s and represents one of the most advanced techniques in immunological analysis [1,2]. This unique specificity makes them indispensable in clinical laboratories, hospitals, and scientific research, where they are used for the diagnosis, monitoring, and prognosis of a wide range of diseases, including infectious, autoimmune, and malignant conditions [3,4]. In recent decades, advances in immunoassay technology have enabled the development of methods that offer faster analysis speeds and the capacity to process large numbers of samples within a short time frame. This progress has significantly reduced the time needed to establish a diagnosis and enabled more efficient treatment. Giagu et al. [5] emphasize that the transition from theoretical foundations to routine laboratory practice in CLIA methods carries several technical challenges, particularly

in signal control and measurement accuracy. Among the most significant technological innovations in this field is chemiluminescent immunoassay (CLIA), which appeared in the early 1980s and quickly established itself as a superior technique [6,7]. CLIA is based on the principle of light (photon) emission during a chemical reaction, allowing for extremely precise and sensitive detection. Unlike older methods such as ELISA (Enzyme-Linked Immunosorbent Assay), CLIA is capable of detecting very low concentrations of analytes in a sample, which is critical for the early diagnosis of infectious diseases. Its high specificity and sensitivity reduce the risk of false-positive or false-negative results, ensuring the reliability of the diagnostic process [8,9]. Due to these advantages, CLIA technology has become the standard in microbiological diagnostics, finding wide application in the detection of viruses, bacteria, and parasites. Premnath and Zubair explain that CLIA methods represent a bridge between classical enzyme immunoassays and highly sensitive luminescent technologies [10].

Modern automated CLIA systems, such as the MAGLUMI X3 analyzer, have further enhanced laboratory operations. These devices are designed for high throughput, allowing for the simultaneous analysis of a large number of samples with minimal human intervention. They are also equipped with advanced self-check and error detection systems, ensuring continuous operation and minimizing the risk of errors in both the pre-analytical and analytical phases [11,12]. Analyzers such as the MAGLUMI X3 offer a wide test panel for the diagnosis of infectious diseases, including tests for Hepatitis B and C, HIV, rubella, and *Mycoplasma pneumoniae*, making them indispensable tools in modern diagnostics and screening [4].

Aim of the Study: To present and analyze the CLIA method and its application in the screening and diagnosis of infectious diseases, with special emphasis on the potential use of the MAGLUMI X3 analyzer and its test panels in microbiology.

Chemiluminescent Immunoassay (CLIA)

CLIA is based on the interaction between antigen and antibody, with signal detection carried out through chemiluminescent reactions [3]. The solid-phase surface in CLIA tests is usually coated with the target antigen or antibody. After adding the sample containing the analyte, a chemiluminescent-labeled secondary antibody is added, which binds to the analyte, forming a complex. This is a highly sensitive and specific detection method that allows for the detection of very low concentrations of substances. Detection is performed by measuring the light intensity (photons) produced by the reaction of the complex with the luminescent substrate [4].

CLIA can be divided into direct and indirect types. The direct method relies on chemical oxidation, while the indirect method uses enzyme labels such as alkaline phosphatase (AP) and horseradish peroxidase (HRP). Xu and colleagues have thoroughly described the indirect competitive CLIA (ic-CLEIA), emphasizing its importance in the quantification of biomolecules [13]. According to the type of solid phase, CLIA is classified into microplate and microparticle formats.

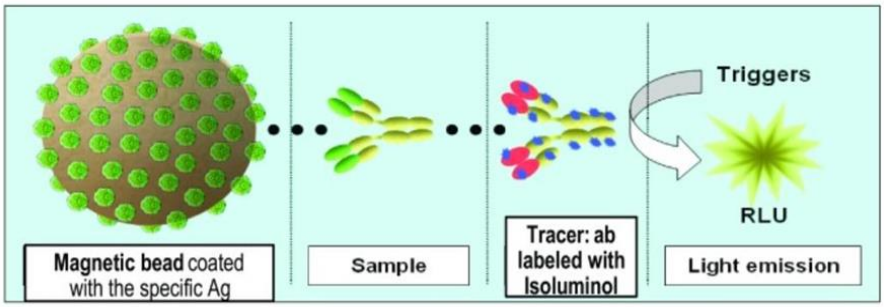

Figure 1. CLIA in the field of autoimmunity [28].

The three most common CLIA methods are:

- Direct Chemiluminescent Immunoassay (CLIA): Uses competitive techniques for the detection of small antigens.
- Chemiluminescent Enzyme Immunoassay (CLEIA): Enzymes bound to antigens or antibodies react with luminescent substrates.
- Electrochemiluminescent Immunoassay (ECLIA): Uses the chemiluminescent agent tris(bipyridine)ruthenium and an electric field to generate chemical luminescence [7].

Principle of Chemiluminescent Immunoassay

To establish a chemiluminescent immunoassay methodology with high specificity and sensitivity, CLIA can be developed by optimizing the sample volume, antibody concentration, and incubation time (Figure 3). Darwish et al. [14]. demonstrated that sensitivity can be significantly increased by introducing a dual enzyme cycle. Kim et al. [15] developed luminol/GO-AuNP hybrid systems for CLIA, confirming the innovative capabilities of the method.

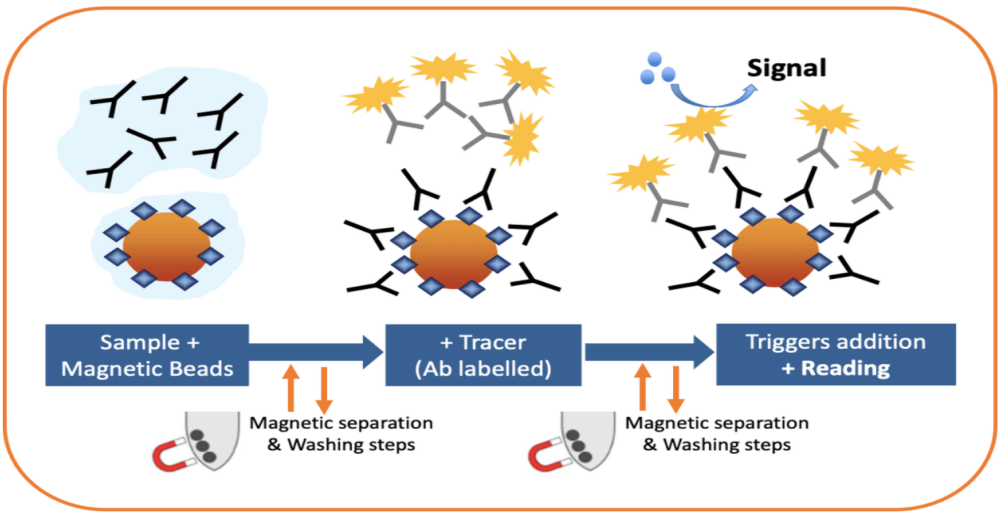

Figure 2. Separation by magnetic particles [29].

Regarding the advantages of this method, Wu et al [16], emphasize that CLIA offers exceptional precision and reproducibility, making it suitable for routine clinical use. Zhu et al. [17] highlighted the importance of automated CLIA systems for rapid and reliable laboratory diagnostics, while Wang et al. [18] demonstrated the use of CLIA in rapid POCT systems, emphasizing the method's advantage in decentralized diagnostics.

Additionally, the method has significant applications in microbiology. Yin et al. [19] applied CLIA for the detection of neutralizing antibodies against SARS-CoV-2, confirming the importance of the method in virology. Zhu et al. [20] developed the CLEIA format for the diagnosis of dengue virus, emphasizing the versatility of the method. Liu et al. [21] demonstrated the use of magnetic particle-based CLIA for the quantification of homocysteine, extending its applicability beyond infectious diseases.

Standard CLIA protocol:

1. Antigen solutions in coating buffer are first applied to the plates. In the sandwich method, the total antibody concentration is determined by coating the wells with an antibody solution that serves as the capture antibody. After sealing, the plates are incubated at an appropriate temperature.
2. The plates are washed with buffer before adding the sample to each well, and the plates are then left to incubate at room temperature.
3. Each well is then filled with monoclonal antibody, and the procedure is repeated.
4. After washing the wells, an increased volume of chemiluminescent substrate solution is added to assess peroxidase activity, and the luminescent signals are read.

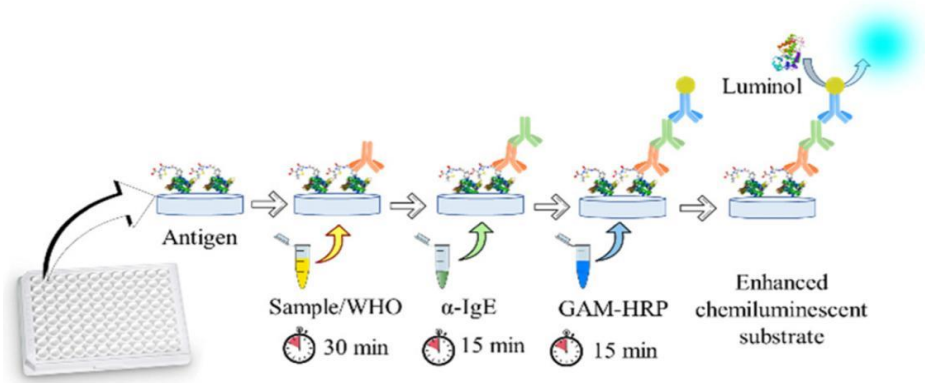

Figure 3. Schematic diagram of CLIA in a 96-well microplate [3].

Methodology

For the purpose of preparing this review article, a systematic search of the scientific literature was conducted to identify relevant studies and review articles related to the application of chemiluminescent immunoassay (CLIA) in microbiology. The search was performed in the PubMed, Google Scholar, and ResearchGate databases using combinations of English keywords such as: chemiluminescence, immunoassay, infectious diseases, microbiology, comparative methods, screening, diagnosis. MeSH terms were utilized to retrieve articles specifically related to the proposed topic. This search identified 1,470 original scientific and review articles published within the last five years. Based on the abstract screening, nine key studies were selected for the purposes of this review (Table 1).

Descriptions of the results and conclusions of the most significant studies analyzed in this paper:

- Comparison of CLIA and ELISA for IgM antibody detection [12]: The study aimed to compare the chemiluminescent immunoassay (Liaison, DiaSorin) and enzyme-linked immunosorbent assay (ELISA) (Euroimmun) for the detection of IgM antibodies against several viral infections. The results showed that CLIA was perfectly comparable to ELISA for detecting IgM antibodies against measles (κ=0.86) and mumps (κ=0.92). For rubella, CMV, EBV, and HHV-1 and -2, the agreement was moderate, but PABAK (prevalence-adjusted bias-adjusted kappa) showed improved concordance. It was concluded that both tests can be used comparably depending on laboratory conditions.
- Comparison of new CLIA and passive agglutination for the diagnosis of *M. pneumoniae* in children [22]: This comparative study included 291 children. Results showed that CLIA had high sensitivity (86.7%) and specificity (80.09%) compared to the PA test, which is considered the diagnostic standard. There was high consistency (76.8%) between both methods with a Kappa coefficient of 0.80. It was concluded that CLIA is a reliable and rapid method and a promising alternative to the PA test.
- Comparison of CLIA, ELISA, and passive agglutination for the diagnosis of *M. pneumoniae* [8]: In this study, researchers compared CLIA, ELISA, and passive agglutination in 280 patients. CLIA and ELISA showed higher sensitivity compared to the PA test. CLIA showed high agreement with ELISA (over 88%) and higher Kappa coefficients. The authors concluded that CLIA has greater specificity and sensitivity for detecting IgM and IgG antibodies and should be used for clinical diagnosis of *M. pneumoniae* infection.
- Comparative evaluation of three diagnostic tests for detecting monkeypox virus in humans [23]: This study aimed to compare three tests for detecting the monkeypox virus. It was shown that the MAGLUMI® monkeypox virus Ag test (CLIA) had high sensitivity, with a limit of detection (LoD) of 0.500 pg/mL. The specificity of all three tests was 100%. The study provides valuable insight into the clinical application of monkeypox tests.

- Chemiluminescent immunoassay for the detection of mumps virus antibodies [6]: This study evaluated the performance of the CLIA test for detecting mumps virus antibodies. Testing was performed on 40 serum samples. There was good correlation between the CLIA method, enzymatic fluorescence assay, and hemagglutination inhibition test. The sensitivity of the CLIA test was approximately 10 times higher than that of the hemagglutination inhibition test.
- Evaluation of the performance of MAGLUMI chemiluminescent immunoassay for hepatitis B surface antigen (HBsAg) detection [24]: This study assessed the performance of the MAGLUMI test for HBsAg detection. The diagnostic sensitivity of the test was 100%, with better sensitivity for seroconversion than the reference ARCHITECT HBsAg test. The test showed high reproducibility, a LoD of 0.05 IU/mL, and excellent linearity, confirming that MAGLUMI HBsAg (CLIA) is a highly sensitive and reliable test.
- Clinical performance of the MAGLUMI Anti-HCV (CLIA) test for the detection of hepatitis C virus antibodies [25]: In this study, the authors evaluated the clinical performance of the MAGLUMI Anti-HCV (CLIA) test. Results showed that the test specificity was 99.75% in blood donors and 100% in hospitalized patients, while sensitivity was 100%. It was concluded that the performance of the MAGLUMI Anti-HCV (CLIA) test is excellent, making it suitable for infection diagnosis.
- Evaluation of the MAGLUMI HIV Ab/Ag Combi test for HIV infection detection [26]: This study assessed the performance of the MAGLUMI HIV Ab/Ag Combi test. Results showed high specificity (99.85% in blood donors and 100% in patients) and sensitivity (100%). The sensitivity for seroconversion was comparable to the reference Architect test, confirming that the MAGLUMI HIV Ab/Ag Combi test is reliable for HIV infection screening and diagnosis.
- Evaluation of two chemiluminescence-based platforms for detecting anti-rubella IgG antibodies [27]: This study evaluated the performance of the ARCHITECT I2000SR and MAGLUMI 800 platforms for rubella diagnosis. Both platforms showed good precision and agreement with the reference Cobas e601 system. The authors concluded that these platforms are suitable for routine diagnostics, noting that results should not be extrapolated from one platform to another without additional validation.

Table 1: Overview of relevant studies on the application of the CLIA method

Authors, Title of the Paper, Year	Type of Study	Study Objective	Materials and Methods	Most Significant Results
Runal et al. [12], 2022.	Comparative Study	Comparison of CLIA and ELISA tests for the detection of IgM antibodies.	A total of 345 samples were used. The methods were compared using kappa statistics.	A high agreement (kappa 0.95) was established between CLIA and ELISA, indicating excellent reliability of the CLIA method.
Shiyi et al. [22], 2020.	Comparative Study	Comparison of CLIA and PA methods for the diagnosis of *M. pneumoniae* infection in children.	Testing of serum from 291 children. Cohen's kappa was used to assess agreement.	The CLIA method showed a higher positivity rate for IgM antibodies. The overall agreement rate was 98.6%, with a kappa value of 0.963, indicating almost perfect agreement.
Chen et al. [8], 2018.	Comparative Study	Comparison of CLIA, ELISA, and passive agglutination for the diagnosis of *M. pneumoniae* infection.	Three methods were used on samples from 280 patients.	CLIA had a higher positivity rate for *Mycoplasma pneumoniae* IgM antibodies (27.5%) compared to ELISA (13.6%) and PA (10.4%).
Qu et al. [23], 2024.	Comparative Study	Comparison of three diagnostic tests, including the CLIA method, for the detection of the monkeypox virus.	Use of different tests on patient samples.	The CLIA method demonstrated 100% sensitivity and specificity. Excellent agreement (99.7%) was achieved compared to the reference test, with a kappa value of 0.993.
Konishi et al. [6], 1980.	Evaluation Study	Evaluation of the performance of the CLIA test for the detection of antibodies to the mumps virus.	Comparison of CLIA with the enzyme fluorescence test and the hemagglutination inhibition test on 40 serum samples.	The CLIA test proved to be more reliable, with higher sensitivity (95%) and specificity (100%). No cross-reactivity with other related viruses was observed.
Shen et al. [24], 2024.	Evaluation Study	Assessment of the performance of the MAGLUMI test for the detection of HBsAg.	Comparison of MAGLUMI CLIA with ARCHITECT HBsAg on 411 positive and 5,312 negative samples.	The MAGLUMI HBsAg test showed a sensitivity of 99.76% and specificity of 99.85%. The test is reliable for routine diagnosis of HBsAg.
Li and al. [25], 2023.	Evaluation Study	Clinical evaluation of the MAGLUMI Anti-HCV (CLIA) test.	Testing of the MAGLUMI Anti-HCV test on 5,258 samples from blood donors and patients.	The MAGLUMI Anti-HCV CLIA test is highly sensitive (100%) and specific (99.96%), suitable for routine screening.
Wang et al. [26], 2024.	Evaluation Study	Evaluation of the MAGLUMI HIV Ab/Ag combi test for the detection of HIV infection.	The MAGLUMI HIV Ab/Ag combi test was used on samples from 5,057 blood donors and 213 patients.	The MAGLUMI HIV Ab/Ag combi test demonstrated 100% sensitivity and 99.96% specificity, making it highly effective for screening and diagnosis.
Dembele and al. [27], 2020.	Evaluation Study	Assessment of the performance of the ARCHITECT I2000SR and MAGLUMI 800 platforms for routine diagnosis of serological rubella.	Samples from 113 pregnant women were tested. The performance of the Architect I2000SR and Maglumi 800 platforms for the detection of rubella IgG was analyzed.	Both platforms demonstrated good analytical performance. Sensitivity was 97.53% for Architect and 96.29% for Maglumi. Both are suitable for routine serological diagnosis.

The results of the review of nine relevant studies presented in this paper clearly indicate the superiority and growing application of chemiluminescent immunoassay (CLIA) in modern microbiology. Through analysis of comparative and evaluative studies, the key advantages of the CLIA method have been confirmed, including high sensitivity, specificity, speed, and reliability, enabling rapid and accurate diagnosis of a wide range of infectious diseases.

Studies comparing CLIA with more traditional methods, such as enzyme-linked immunosorbent assay (ELISA) and passive agglutination (PA), consistently showed that CLIA offers improved performance. For example, in the diagnosis of infections caused by *M. pneumoniae*, CLIA tests demonstrated significantly higher sensitivity and specificity compared to ELISA and PA methods [22,8]. Similarly, a study showed that CLIA was perfectly comparable to ELISA for the detection of antibodies against measles and mumps viruses [12], while in the detection of monkeypox, the MAGLUMI® CLIA test showed high sensitivity with a low detection limit [23]. These findings confirm that CLIA not only matches but in many cases surpasses other methods, providing clinicians with more reliable results for decision-making.

A particularly significant part of the analysis concerns the evaluation of specific CLIA platforms such as MAGLUMI and ARCHITECT. Studies have confirmed the outstanding performance of these systems in detecting key viral markers. For instance, the MAGLUMI HBsAg test showed 100% diagnostic sensitivity in early detection of hepatitis B virus infection [24], while the MAGLUMI Anti-HCV test also demonstrated high sensitivity and specificity in detecting antibodies against hepatitis C virus [25]. Furthermore, the MAGLUMI HIV Ab/Ag Combi test proved its reliability in HIV screening with high sensitivity and specificity, making it comparable to reference tests and ideal for early diagnosis [26]. These studies highlight the importance of modern, automated platforms that enable rapid processing of a large number of samples with consistently high precision. Although most studies confirmed the superiority of the CLIA method, it is important to note that results may vary between different platforms. A study comparing the ARCHITECT I2000SR and MAGLUMI 800 platforms for rubella diagnosis concluded that both are suitable for routine use but emphasized that results from one platform should not be extrapolated to another without additional validation [27].

Conclusion

Based on the review and analysis of nine key studies, it can be concluded that chemiluminescent immunoassay (CLIA) represents one of the most advanced and reliable methods in modern microbiological diagnostics. Its advantages in terms of high sensitivity, specificity, and speed make it an indispensable tool for early screening and precise diagnosis of a wide range of infectious diseases, including infections caused by hepatitis viruses, HIV, measles, mumps, and the bacterium *M. pneumoniae*.

Modern, automated CLIA systems, such as the MAGLUMI and ARCHITECT platforms, have demonstrated exceptional efficiency and reliability in clinical studies, confirming their role in advancing laboratory practice. Although it is necessary to

consider the specific characteristics of different platforms, the CLIA method as a whole significantly contributes to faster and more accurate diagnosis, which directly impacts timely treatment and improved patient outcomes.

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METODA HEMILUMINESCENCIJE U MIKROBIOLOGIJI S OSVRTOM NA MOGUĆNOSTI SKRININGA I DIJAGNOSTIKE ZARAZNIH OBOLJENJA

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Sažetak. *Hemiluminiscencijsko imunotestiranje (CLIA) je visoko osjetljiva i specifična metoda koja se koristi u mikrobiološkim laboratorijama za dijagnostiku infektivnih bolesti. CLIA koristi hemiluminiscentne supstrate i enzime, poput peroksidaze hrena (HRP) i alkalne fosfataze (AP), za detekciju antigena ili antitijela putem svjetlosnog signala. Postoji nekoliko formata CLIA testova, uključujući direktni hemiluminiscencijski imunotest (CLIA), hemiluminiscencijski enzimski imunotest (CLEIA) i elektrohemiluminiscencijski imunotest (ECLIA), koji se koriste prema vrsti analita i potrebama ispitivanja. Savremeni uređaji zasnovani na CLIA metodi omogućavaju preciznu i pouzdanu detekciju brojnih infektivnih agenasa, uključujući viruse, bakterije i parazite. Ovi uređaji su opremljeni sistemima za automatsku detekciju grešaka u pipetiranju i ispitivanju uzoraka, čime omogućavaju neprekidan rad i visoku efikasnost. Primjena CLIA metode u mikrobiologiji ključna je za preciznu dijagnozu i praćenje infektivnih bolesti. CLIA se koristi za dijagnostiku širokog spektra zaraznih bolesti izazvanih mikroorganizmima, uključujući viruse poput HIV-a, bakterije i parazite. Ova metoda omogućava visoko preciznu identifikaciju patogena, što je od velike važnosti za brzu i tačnu dijagnozu te odgovarajuće liječenje.*

Ključne riječi*: hemiluminiscencija, imunotestiranje, mikrobiologija, skrining, enzimi*