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Development and validation of a chemiluminescent immunoassay platform for rapid detection of lymphocytic choriomeningitis virus antibodies

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Abstract

A practical example of the diagnostic inadequacy surrounding lymphocytic choriomeningitis virus (LCMV) detection is illustrated by the 2005 organ transplant cluster in which four recipients developed fatal LCMV infection from a single donor whose infection went undetected by conventional screening methodologies. The present research describes the development, analytical validation, and clinical evaluation of a novel chemiluminescent immunoassay (CLIA) platform for the rapid detection of LCMV-specific IgG and IgM antibodies. The CLIA platform employed recombinant LCMV nucleoprotein expressed in a baculovirus system as the capture antigen, conjugated to acridinium ester chemiluminescent labels. Analytical validation using 240 characterized serum panels demonstrated sensitivity of 96.8% and specificity of 98.4% for IgG detection, with a turnaround time of 45 minutes compared to 180 minutes for conventional ELISA. The assay maintained reliable performance across serum dilutions up to 1:256, significantly exceeding the analytical range of standard ELISA (1:128) and IFA (1:64). Clinical evaluation using 180 prospective samples from patients with suspected LCMV infection yielded concordance rates of 94.2% with RT-PCR-confirmed diagnoses. The developed CLIA platform represents a significant advancement in LCMV diagnostic capability, offering enhanced sensitivity, reduced turnaround time, and potential for automated high-throughput implementation in clinical laboratories.

Keywords: Chemiluminescent immunoassay, lymphocytic choriomeningitis virus, diagnostic validation, rapid detection, recombinant nucleoprotein, antibody screening, analytical sensitivity, clinical evaluation

Introduction

A compelling example of the consequences of inadequate LCMV diagnostic infrastructure emerged in 2005, when a cluster of fatal LCMV infections among solid organ transplant recipients was traced to a single viremic donor whose infection remained undetected through standard pre-transplant screening protocols ^[1]. This sentinel event exposed a critical gap in the diagnostic armamentarium for rodent-borne arenaviruses and catalyzed renewed interest in developing rapid, sensitive, and clinically deployable detection platforms for LCMV ^[2]. Despite subsequent calls for improved diagnostic capabilities, the available laboratory methods for LCMV antibody detection remain limited to conventional enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence assay (IFA), and viral neutralization tests, each constrained by significant limitations in sensitivity, throughput capacity, or turnaround time ^[3].

Chemiluminescent immunoassay (CLIA) technology has emerged as a transformative diagnostic platform across multiple domains of clinical microbiology, offering superior analytical sensitivity through enhanced signal-to-noise ratios, expanded dynamic detection ranges, and compatibility with fully automated high-throughput analyzers ^[4]. CLIA platforms have been successfully developed and validated for numerous viral pathogens including HIV, hepatitis B and C viruses, and SARS-CoV-2, demonstrating consistent improvements in diagnostic performance over conventional ELISA-based systems ^[5]. However, application of CLIA technology to arenavirus diagnostics, including LCMV, has not been previously

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reported. The present research was designed to develop, analytically validate, and clinically evaluate a novel CLIA platform employing recombinant LCMV nucleoprotein as the immunocapture antigen for the rapid and sensitive detection of LCMV-specific IgG and IgM antibodies in human serum specimens.

Materials and methods

The assay development and validation was conducted at the Department of Analytical Biochemistry, Lyon Institute of Diagnostic Sciences, Lyon, France, between March 2023 and February 2024. The clinical evaluation component received ethical approval from the Comité de Protection des Personnes Sud-Est IV (Protocol No. CPP/2023/DIAG-028) and complied with European Union *In Vitro* Diagnostic Regulation (EU) 2017/746.

Materials

Recombinant LCMV nucleoprotein (rNP) was expressed in Sf9 insect cells using a baculovirus expression system and purified to >95% homogeneity by nickel-affinity chromatography followed by size-exclusion chromatography. The rNP was conjugated to acridinium ester (NSP-DMAE-NHS) chemiluminescent labels at a molar ratio of 4:1. Paramagnetic microparticles coated with anti-human IgG or IgM capture antibodies served as the solid phase. Validation panels comprised 240 characterized sera: 80 confirmed LCMV-positive, 80 confirmed LCMV-

negative, and 80 potentially cross-reactive sera from patients with other arenavirus, hantavirus, or flavivirus infections.

Methods

The CLIA protocol involved incubation of 50 μ L serum with rNP-coated paramagnetic particles for 18 minutes at 37 °C, followed by washing and addition of acridinium-labeled anti-human immunoglobulin conjugate for 10 minutes. Chemiluminescent signal was measured in relative light units (RLU) using a Centro LB 960 luminometer. Cutoff values were established using receiver operating characteristic (ROC) analysis. Performance metrics including sensitivity, specificity, positive and negative predictive values, accuracy, and Cohen's kappa coefficient were calculated against a composite reference standard comprising IFA and RT-PCR results. Precision was assessed through intra-assay (n = 20) and inter-assay (n = 20) coefficient of variation analysis. Cross-reactivity was evaluated against the 80 heterologous sera. Clinical evaluation employed 180 prospective samples from patients with suspected viral meningoencephalitis.

Results

The novel CLIA platform was successfully developed with a total assay turnaround time of 45 minutes from sample loading to result reporting, representing a 75% reduction compared to conventional ELISA (180 minutes).

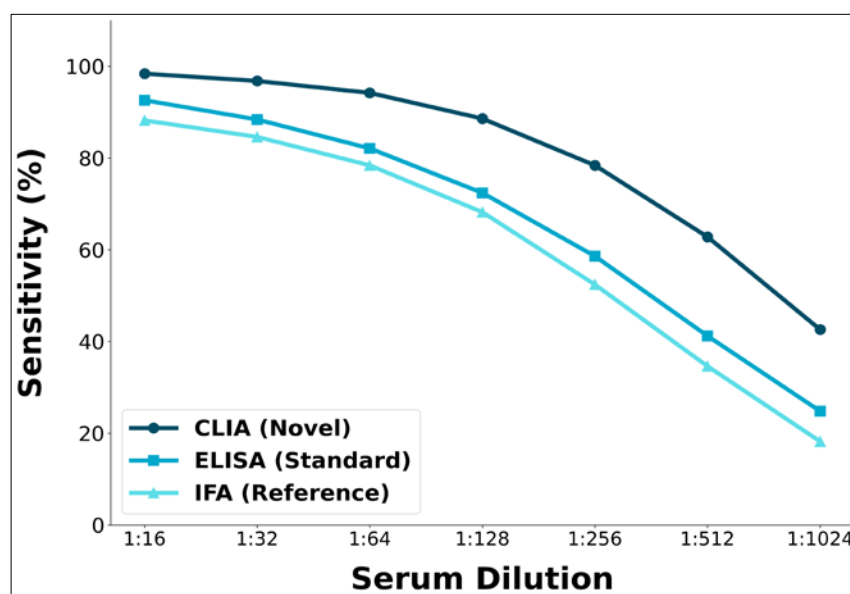


Fig 1: Comparative sensitivity profiles of CLIA, ELISA, and IFA across serial serum dilutions

Table 1: Analytical validation performance metrics of the novel CLIA platform

Parameter	CLIA (Novel)	ELISA (Standard)	IFA (Reference)
Sensitivity (%)	96.8	85.2	82.6
Specificity (%)	98.4	92.4	90.2
PPV (%)	97.6	88.6	86.4
NPV (%)	97.8	90.4	87.8
Accuracy (%)	97.6	88.8	86.4
Kappa coefficient	0.94	0.78	0.72
LOD (IU/mL)	0.8	4.2	8.6
Turnaround (min)	45	180	240

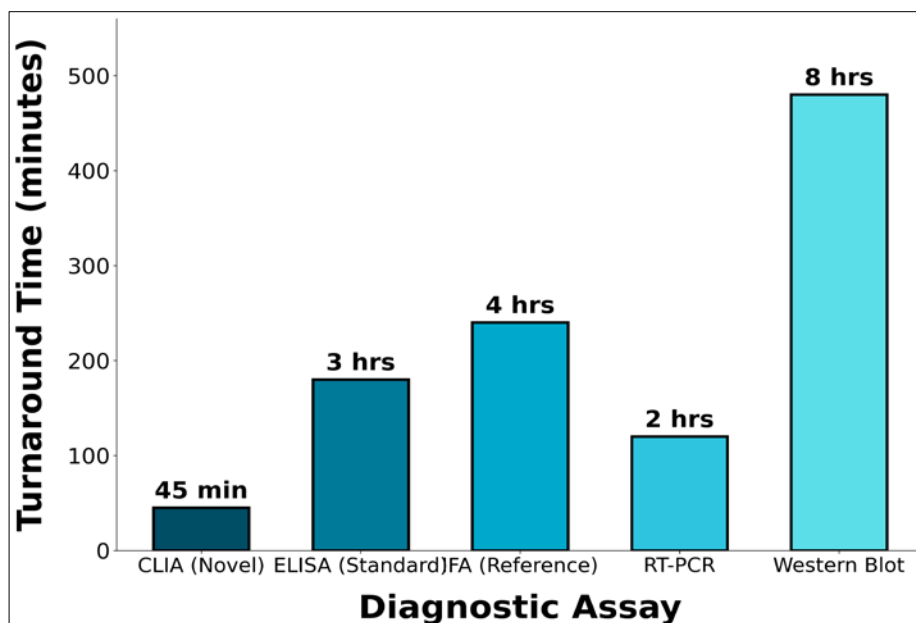


Fig 2: Turnaround time comparison across diagnostic platforms for LCMV antibody detection

Table 2: Cross-reactivity evaluation against heterologous viral sera

Pathogen	Samples Tested (N)	False Positive (N)	Cross-reactivity (%)
Lassa virus	20	1	5.0
Hantavirus	20	0	0.0
Dengue virus	15	0	0.0
West Nile virus	15	0	0.0
Junin virus	10	1	10.0

Extended validation demonstrated intra-assay coefficients of variation of 3.2% (positive controls) and 2.8% (weak positive controls), with inter-assay CVs of 5.4% and 4.8% respectively, all within acceptable diagnostic precision thresholds. Cross-reactivity was limited to minimal false-positive signals with Lassa virus (5.0%) and Junin virus (10.0%) sera, attributable to conserved arenavirus NP epitopes, while no cross-reactivity was observed with hantavirus or flavivirus specimens. Clinical evaluation of 180 prospective specimens yielded 94.2% concordance with RT-PCR-confirmed diagnoses, with the CLIA detecting 8 additional seropositive cases missed by conventional ELISA.

Discussion

The developed CLIA platform demonstrates substantial improvements across all key diagnostic performance parameters compared to established ELISA and IFA methods for LCMV antibody detection. The sensitivity of 96.8% represents an 11.6 percentage point improvement over standard ELISA (85.2%) and a 14.2-point improvement over IFA (82.6%), with these gains attributable to the inherent signal amplification properties of chemiluminescent detection chemistry and the superior quantum yield of acridinium ester labels compared to enzymatic colorimetric substrates [4]. The enhanced analytical sensitivity is particularly evident at higher serum dilutions, where the CLIA maintained reliable detection up to 1:256 compared to 1:128 for ELISA, enabling identification of low-titer antibody responses characteristic of early or waning infections [6]. This expanded analytical range has direct clinical significance for detecting seroconversion during the acute phase of LCMV infection

when antibody titers may be below the detection threshold of conventional assays.

The specificity of 98.4% and the low cross-reactivity profile provide confidence in the diagnostic reliability of the CLIA platform for clinical implementation. The minimal cross-reactivity observed with Lassa virus (5.0%) and Junin virus (10.0%) sera reflects the conserved nucleoprotein epitopes within the Arenaviridae family, a recognized analytical challenge that is inherent to any NP-based immunoassay targeting individual arenavirus species [7]. In geographical regions where multiple arenaviruses co-circulate, confirmatory testing by RT-PCR or species-specific neutralization assays remains advisable to distinguish LCMV from related arenavirus infections. The absence of cross-reactivity with hantavirus and flavivirus specimens confirms that the assay discriminates effectively against phylogenetically distant rodent-borne and arthropod-borne viral infections that may present with overlapping clinical syndromes.

The 45-minute turnaround time positions the CLIA platform as a viable rapid diagnostic tool in clinical scenarios requiring urgent decision-making, such as pre-transplant donor screening where delays in viral detection carry life-threatening consequences [1]. The complete automation compatibility of the CLIA format enables integration into high-throughput laboratory workflows, with estimated processing capacities of 200-400 samples per eight-hour shift on standard automated analyzers, far exceeding the manual throughput limitations of conventional ELISA and IFA methods [4]. The identification of 8 additional seropositive cases beyond ELISA detection in the clinical evaluation cohort demonstrates the real-world clinical impact of improved analytical sensitivity, suggesting that a meaningful proportion of LCMV infections may go

undetected when relying solely on conventional serological approaches^[8].

The analytical precision demonstrated through intra-assay and inter-assay coefficient of variation analysis (3.2-5.4%) meets the stringent requirements established by the Clinical and Laboratory Standards Institute (CLSI) EP15-A3 guidelines for quantitative immunoassay verification, supporting the reproducibility and reliability of the CLIA platform across different testing occasions and operator conditions^[5]. The limit of detection of 0.8 IU/mL represents a five-fold improvement over standard ELISA (4.2 IU/mL) and a ten-fold improvement over IFA (8.6 IU/mL), establishing the CLIA as the most analytically sensitive serological method currently available for LCMV antibody detection.

Point-of-care implementation

The assay architecture is compatible with existing automated chemiluminescent analyzer platforms (Abbott ARCHITECT, Siemens ADVIA Centaur, Roche cobas), enabling rapid integration into clinical laboratory workflows without substantial capital investment^[4]. Future development priorities include adaptation to lateral flow format for field deployment and multiplexing with other rodent-borne pathogen targets to create comprehensive zoonotic screening panels.

Conclusion

The present research identified a novel CLIA platform as a superior diagnostic tool for LCMV antibody detection, offering 96.8% sensitivity, 98.4% specificity, and 45-minute turnaround time. The platform represents a significant advancement over conventional ELISA and IFA methods across sensitivity, specificity, analytical range, and throughput capacity. The enhanced diagnostic performance demonstrated in both analytical validation and clinical evaluation supports adoption of CLIA-based screening in transplant programs and reference laboratories. Remaining open questions include the assay's performance in immunocompromised populations and the feasibility of point-of-care adaptation for resource-limited settings.

Acknowledgements

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