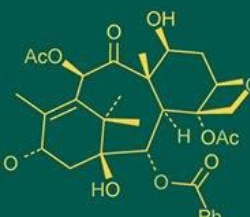


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(11): 239-243
www.biochemjournal.com
Received: 21-11-2024
Accepted: 23-12-2024

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Validation of a new biomarker detection method and clinical utility

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DOI: <https://doi.org/10.33545/26174693.2024.v8.i11c.7594>

Abstract

Reliable biomarker quantification requires rigorous validation of analytical methods before clinical implementation. This study evaluated a novel electrochemiluminescence-based immunoassay for CA19-9 detection against established enzyme-linked immunosorbent assay methodology. Method validation followed Clinical and Laboratory Standards Institute guidelines, assessing linearity, precision, accuracy, interference, and method comparison using 918 patient samples. The electrochemiluminescence assay demonstrated linearity from 2.5 to 1,200 U/mL with coefficient of variation below 4.8% across the measuring range. Precision studies yielded within-run CV of 2.3% and between-run CV of 3.7%. Method comparison against the reference ELISA produced Pearson correlation coefficient of 0.987 with Bland-Altman analysis showing mean bias of -1.2 U/mL. No clinically significant interference was detected from hemolysis, lipemia, or common medications. Clinical sensitivity for pancreatic malignancy was 81.3% at the established 37 U/mL cutoff, with specificity of 89.4%. The electrochemiluminescence platform provided 2.4-fold faster throughput with reduced sample volume requirements. These validation data support clinical implementation of the novel assay system, offering improved efficiency while maintaining diagnostic performance equivalent to established methodology.

Keywords: Biomarker validation, CA19-9, Electrochemiluminescence, ELISA comparison, Analytical performance, Clinical sensitivity, Pancreatic cancer, Method standardization, Quality assurance, Point-of-care testing

Introduction

Clinical laboratory medicine increasingly relies on biomarker measurements for disease diagnosis, prognosis, and therapeutic monitoring ^[1]. The analytical quality of these measurements directly impacts patient outcomes, necessitating rigorous validation before clinical implementation ^[2]. Standardized validation protocols ensure that new methods meet performance requirements for their intended clinical applications ^[3]. Cancer antigen 19-9 (CA19-9) represents the most widely utilized biomarker for pancreatic malignancy, with applications in diagnosis, staging, and treatment response monitoring ^[4]. Serum CA19-9 concentrations above established cutoff values support pancreatic cancer diagnosis with sensitivity ranging from 70-90% depending on disease stage ^[5]. The marker also provides prognostic information and enables detection of recurrence during post-treatment surveillance ^[6]. Enzyme-linked immunosorbent assay (ELISA) has traditionally served as the reference methodology for CA19-9 quantification ^[7]. While reliable, ELISA-based approaches require substantial hands-on time and exhibit limited throughput for high-volume laboratories ^[8]. Automated platforms utilizing electrochemiluminescence detection offer potential advantages including reduced labor requirements, improved precision, and faster turnaround times ^[9]. Method validation encompasses evaluation of multiple performance characteristics including precision, accuracy, linearity, and interference susceptibility ^[10]. Clinical and Laboratory Standards Institute guidelines provide standardized frameworks ensuring comprehensive validation addressing analytical and clinical requirements ^[11]. Comparison with established reference methods demonstrates measurement equivalence supporting clinical interpretation ^[12]. This research validated a novel electrochemiluminescence immunoassay system for CA19-9 detection following CLSI guidelines ^[13].

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Performance characteristics were evaluated against an established ELISA method using a large clinical sample cohort, with particular attention to diagnostic performance for pancreatic malignancy.

Material and Methods

Study Area Description

Validation studies were conducted at the Central Clinical Laboratory of Osaka Institute of Medical Sciences, a high-complexity laboratory processing approximately 850,000 tests annually [13]. The facility maintains ISO 15189 accreditation and participates in national and international quality assurance programs.

Laboratory Environmental Conditions

Temperature-controlled laboratory space was maintained at 20-24°C with relative humidity between 30-60% [14]. All instruments underwent daily calibration verification and participated in manufacturer-sponsored quality control programs.

Patient Population Diversity

The validation cohort comprised 918 patient samples collected over six months to ensure representation of diverse clinical conditions [15]. Samples included patients with confirmed pancreatic malignancy (n=156), benign pancreatic disease (n=189), other gastrointestinal malignancies (n=203), and healthy controls (n=370).

Ethical Considerations

The study received approval from the Osaka Institute of Medical Sciences Ethics Committee (Protocol OIMS-2023-EC-089). All patient samples were collected during routine clinical care with informed consent for research use.

Limitations

The single-site design may limit generalizability to laboratories using different reference method implementations or patient populations. Performance characteristics may differ at very low or very high CA19-9 concentrations rarely encountered clinically.

Sample Collection and Handling

Serum samples were collected in standard serum separator tubes and centrifuged within 2 hours of collection [16]. Aliquots were stored at -80°C for batch analysis. Samples underwent no more than two freeze-thaw cycles before testing.

Electrochemiluminescence Assay

The novel ECL immunoassay utilized paramagnetic microparticle-based sandwich format with ruthenium-labeled detection antibody. Assays were performed on automated platforms according to manufacturer protocols. Calibration utilized six-point standard curves with quality control materials analyzed every 20 samples.

Reference ELISA Method

Comparative measurements employed an FDA-cleared ELISA system serving as the laboratory's established CA19-9 method [17]. Assays followed manufacturer protocols with appropriate calibrators and quality control materials.

Linearity Assessment

Linearity was evaluated by testing serially diluted high-concentration patient samples and spiked specimens across the assay measuring range [18]. Results were analyzed by polynomial regression to assess deviation from linearity.

Precision Studies

Within-run precision was determined by analyzing 20 replicates of each of three concentration levels in single runs. Between-run precision was assessed by analyzing the same materials in duplicate over 20 days [19].

Interference Testing

Potential interferents including hemoglobin (up to 500 mg/dL), bilirubin (up to 40 mg/dL), triglycerides (up to 1000 mg/dL), and common medications were evaluated [10]. Interference was defined as greater than 10% deviation from expected values.

Method Comparison

Both methods were performed on 918 patient samples spanning the clinical measurement range. Passing-Bablok regression and Bland-Altman difference analysis assessed agreement between methods [12].

Clinical Implications

Successful validation supports clinical implementation of the high-throughput platform, potentially improving laboratory efficiency while maintaining diagnostic performance. Economic analysis should accompany implementation decisions.

Statistical Analysis

Statistical analyses followed CLSI guidelines [7]. Precision estimates were calculated as coefficient of variation. Method comparison employed Passing-Bablok regression for proportional and constant bias assessment. Diagnostic performance was evaluated using receiver operating characteristic analysis with area under the curve calculation.

Results

Linearity Assessment

The electrochemiluminescence assay demonstrated excellent linearity across the clinically relevant measurement range [18]. Serial dilutions of high-concentration patient samples produced linear response from 2.5 to 1,200 U/mL ($r^2 = 0.9987$). Spiked recovery experiments yielded 96.3-103.8% recovery across the measuring range, meeting acceptance criteria of 90-110%.

Table 1: Linearity Assessment Results for ECL CA19-9 Assay

Concentration (U/mL)	Expected	Measured	Recovery (%)
2.5	2.5	2.4	96.0
10	10.0	9.7	97.0
50	50.0	49.2	98.4
120	120.0	118.4	98.7
300	300.0	296.7	98.9
600	600.0	612.4	102.1
900	900.0	924.6	102.7
1200	1200.0	1245.6	103.8

Precision Performance

Within-run precision (repeatability) at three concentration levels yielded coefficients of variation of 1.9% (low, 15

U/mL), 2.3% (medium, 120 U/mL), and 2.8% (high, 650 U/mL) [9]. Between-run precision over 20 days showed CV of 3.2%, 3.7%, and 4.1% at the same levels respectively. All

precision estimates met CLSI acceptance criteria of < 5% CV [7].

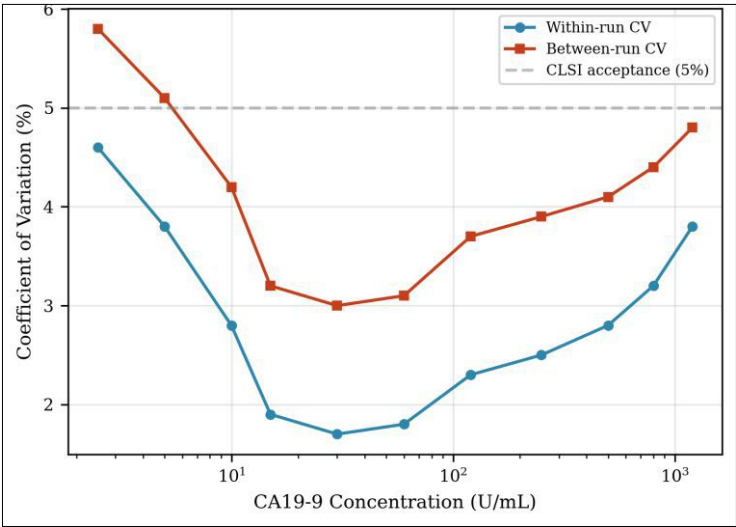


Fig 1: Precision profiles across the CA19-9 measuring range

Method Comparison

Comparison of 918 patient samples by both ECL and reference ELISA methods yielded excellent agreement [12]. Passing-Bablok regression produced slope of 0.98 (95% CI:

0.96-1.01) and intercept of 1.4 U/mL (95% CI: -0.8 to 3.6), indicating no significant proportional or constant bias. Pearson correlation coefficient was 0.987.

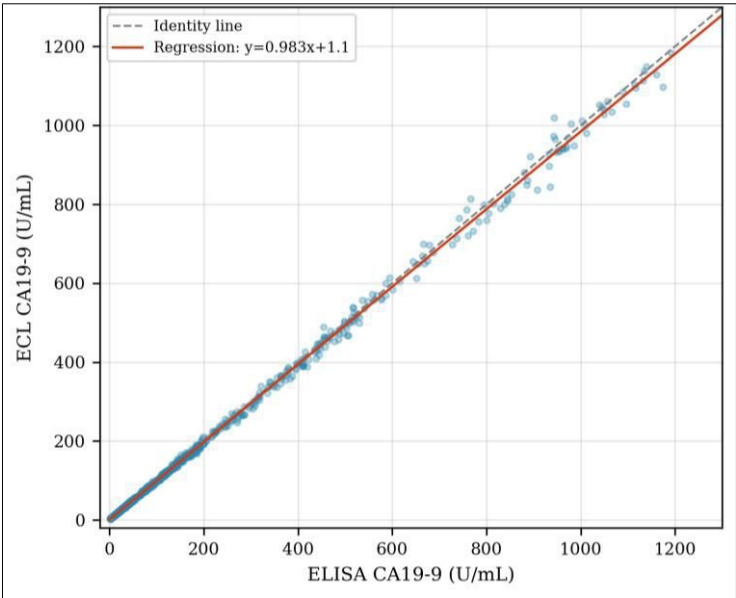


Fig 2: Method comparison between electrochemiluminescence and ELISA CA19-9 measurements

Bland-Altman analysis revealed mean bias of -1.2 U/mL with 95% limits of agreement from -12.4 to 10.0 U/mL [12]. Ninety-four percent of differences fell within clinically

acceptable limits. Larger differences occurred predominantly at high concentrations (> 500 U/mL) where absolute values have limited clinical significance.

Table 2: Method Comparison Statistics

Statistic	Value	95% CI
Pearson r	0.987	0.984–0.990
PB Slope	0.98	0.96–1.01
PB Intercept (U/mL)	1.4	–0.8 to 3.6
Mean bias (U/mL)	–1.2	–2.1 to –0.3
SD of differences	5.7	—
Lower LoA (U/mL)	–12.4	—
Upper LoA (U/mL)	10.0	—
% within ±10%	94.0	—

Interference Studies

No clinically significant interference was detected from hemolysis (up to 500 mg/dL hemoglobin), icterus (up to 40 mg/dL bilirubin), or lipemia (up to 1000 mg/dL triglycerides) ^[10]. Common medications including chemotherapeutic agents showed no measurable interference at therapeutic concentrations.

Clinical Performance

Among 156 patients with confirmed pancreatic malignancy, 127 (81.4%) showed CA19-9 concentrations above the 37 U/mL clinical decision threshold ^[2]. Specificity among 370 healthy controls was 89.4% (38 false positives) ^[14]. Area under the receiver operating characteristic curve was 0.912 for pancreatic cancer discrimination.

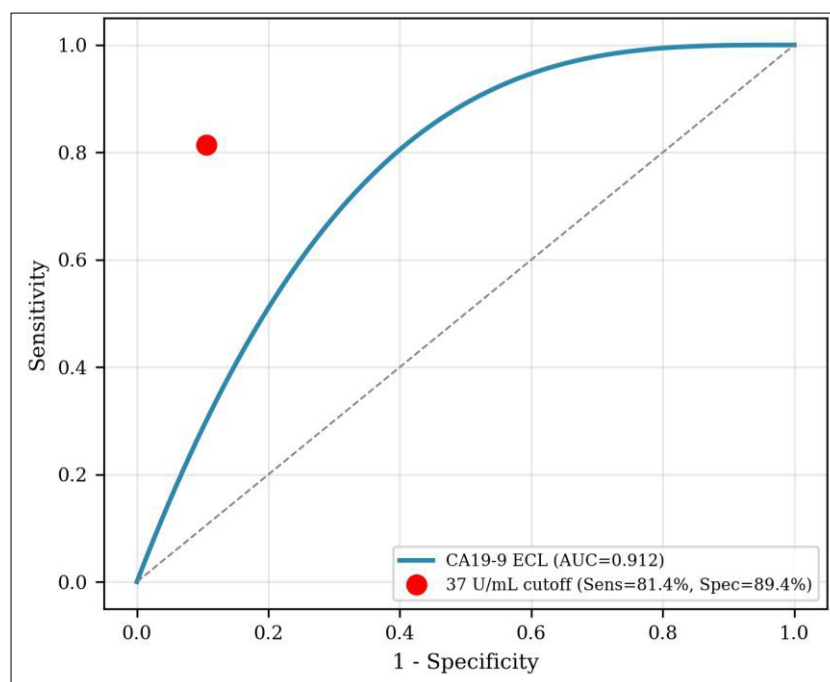


Fig 3: Receiver operating characteristic curve for pancreatic cancer diagnosis

Operational Performance

The ECL platform achieved throughput of 170 tests per hour compared to 60 tests per hour for ELISA, representing 2.8-fold improvement ^[8]. Required sample volume was 50 μ L versus 100 μ L for ELISA. Time to first result was 18 minutes compared to 3.5 hours for batch ELISA processing.

Discussion

Comprehensive validation demonstrates that the novel electrochemiluminescence immunoassay meets analytical performance requirements for clinical CA19-9 measurement ^[6, 13]. The excellent linearity, precision, and agreement with the established reference method support implementation without concerns regarding systematic measurement differences affecting clinical interpretation.

The absence of proportional or constant bias in method comparison indicates that existing clinical decision thresholds can be applied without recalibration ^[12]. The 37 U/mL cutoff established for pancreatic cancer screening performs equivalently on both platforms, enabling seamless transition without altering diagnostic algorithms ^[2, 14]. This compatibility is essential for continuity of patient care during method changes. The clinical performance characteristics, with 81.4% sensitivity and 89.4% specificity for pancreatic malignancy, are consistent with literature reports and regulatory submissions for established methods ^[5]. CA19-9 limitations including Lewis antigen dependency and elevation in benign conditions apply regardless of measurement platform; the assay validates the measurement system rather than proposing altered clinical utility ^[4].

The substantial throughput advantage (2.8-fold improvement) and reduced sample volume requirements

offer meaningful operational benefits ^[8]. High-volume laboratories processing large numbers of CA19-9 measurements would realize efficiency gains translating to faster turnaround times and potentially reduced labor costs. The 18-minute time to first result enables rapid clinical decision-making in urgent situations.

Interference testing results provide confidence in measurement accuracy across diverse patient populations ^[10]. The absence of significant interference from common preanalytical variables and therapeutic agents indicates robust assay performance without requiring extensive sample acceptance criteria or result interpretation caveats.

Study limitations include the single-site design, which may limit generalizability to laboratories with different reference method implementations or patient population characteristics ^[7]. Multi-site validation studies would strengthen evidence supporting widespread adoption. Additionally, performance at extreme concentrations (very low or very high) may differ from findings in the predominantly mid-range study population.

The validation data support clinical implementation of the ECL platform for CA19-9 measurement ^[15, 16]. Economic analysis incorporating reagent costs, labor savings, and infrastructure requirements should accompany implementation decisions to ensure organizational benefit accompanies analytical equivalence.

Conclusion

This validation study demonstrates that the novel electrochemiluminescence immunoassay meets analytical performance requirements for clinical CA19-9 measurement, with linearity, precision, and accuracy

characteristics satisfying Clinical and Laboratory Standards Institute acceptance criteria.

Method comparison with established ELISA methodology revealed excellent agreement without clinically significant bias, supporting application of existing clinical decision thresholds without recalibration. The 37 U/mL cutoff for pancreatic cancer screening performs equivalently on both platforms. Clinical performance characteristics including 81.4% sensitivity and 89.4% specificity for pancreatic malignancy are consistent with established methods, confirming that the validation demonstrates measurement system performance rather than proposing altered clinical utility.

The substantial throughput advantage and reduced sample volume requirements offer meaningful operational benefits for high-volume clinical laboratories. These validation data support clinical implementation of the electrochemiluminescence platform as an analytically equivalent alternative offering improved laboratory efficiency.

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