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Graphene Nanomaterial for Biosensors - A Boon for Early Cancer Detection: A Systematic Review

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ABSTRACT

Background: Cancer remains the second leading cause of death globally, with approximately 10 million deaths in 2020 according to the World Health Organization [1]. Early detection significantly improves survival rates, with 5-year survival rates exceeding 90% for many cancers when detected at early stages [2]. Conventional diagnostic methods including enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and immunohistochemistry often lack the sensitivity required for detecting low-abundance biomarkers in early-stage cancer [3,4]. Graphene-based nanomaterials have emerged as revolutionary platforms for biosensor development due to their exceptional electrical conductivity (up to 6000 S/cm), large specific surface area (2630 m^2/g), and superior electron transfer properties [5,6].

Objectives: To systematically review and synthesize evidence on graphene, graphene oxide (GO), and reduced graphene oxide (rGO)-based biosensors for early cancer detection, evaluating their analytical performance, clinical validation status, and translational potential for point-of-care applications.

Methods: A comprehensive literature search was conducted across PubMed, Scopus, Web of Science, and IEEE Xplore databases from inception to September 2024. Studies investigating graphene-based biosensors for detecting cancer biomarkers were included. Search terms combined "graphene," "biosensor," and "cancer detection" with appropriate Boolean operators. Two independent reviewers performed screening, data extraction, and quality assessment. Data on biosensor types, target biomarkers, cancer types, limit of detection (LOD), sensitivity, specificity, and clinical validation were extracted and synthesized.

Results: A total of 87 studies met inclusion criteria, encompassing electrochemical (n=52, 59.8%), optical (n=21, 24.1%), field-effect transistor-based (n=10, 11.5%), and other biosensor platforms (n=4, 4.6%). Graphene-based biosensors demonstrated LOD values ranging from 0.1 fg/mL to 100 ng/mL for various cancer biomarkers, representing 10- to 1000-fold improvement over conventional ELISA methods. Breast cancer (n=28), lung cancer (n=19), prostate cancer (n=15), and colorectal cancer (n=12) were most frequently investigated. Protein biomarkers including carcinoembryonic antigen (CEA), prostate-specific antigen (PSA), cancer antigen 125 (CA125), and alpha-fetoprotein (AFP) were detected with LODs as low as 1 fg/mL. MicroRNA detection achieved LODs of 0.1 fM, and circulating tumor cell detection sensitivity reached 1-5 cells/mL. Clinical validation using patient samples was reported in only 23% (n=20) of studies, with sample sizes ranging from 10 to 156 patients.

Conclusions: Graphene-based biosensors demonstrate exceptional analytical performance with ultra-high sensitivity and low detection limits superior to conventional methods. Electrochemical platforms show the most promise for clinical translation due to simplicity, cost-effectiveness, and miniaturization potential. However, significant challenges remain including lack of standardization, limited large-scale clinical validation, regulatory approval pathways, and manufacturing reproducibility. Future research priorities should focus on prospective multicenter clinical trials, point-of-care device development, multiplexed detection systems, and establishing standardization protocols for clinical implementation.

Keywords: *Graphene, Graphene oxide, Reduced graphene oxide, Biosensors, Cancer biomarkers, Early detection, Electrochemical sensors, Point-of-care diagnostics*

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1. INTRODUCTION

1.1 Background and Context

Cancer represents one of the most significant public health challenges globally, with the International Agency for Research on Cancer (IARC) reporting 19.3 million new cancer cases and nearly 10 million cancer deaths worldwide in 2020 [7]. Projections indicate that cancer incidence will reach approximately 28.4 million cases by 2040, representing a 47% increase from 2020 levels [7]. The economic burden is equally staggering, with global cancer costs estimated at $1.16 trillion annually [8].

The cornerstone of improving cancer outcomes lies in early detection. Five-year survival rates for breast cancer exceed 99% when detected at localized stages (Stage I) but decrease dramatically to 27% for distant metastatic disease [9]. Similarly, colorectal cancer demonstrates 90% five-year survival for early-stage detection versus 14% for advanced stages [10]. These statistics underscore the critical importance of developing highly sensitive diagnostic tools capable of detecting cancer at its earliest, most treatable stages.

Current gold standard diagnostic methods include imaging techniques (computed tomography, magnetic resonance imaging, positron emission tomography), tissue biopsy with histopathological examination, and biochemical assays such as ELISA, immunohistochemistry, and molecular diagnostics [11,12]. However, these methods have inherent limitations. Imaging techniques often detect tumors only after they have grown to substantial sizes (typically >1 cm^3, containing approximately 10^9 cells) [13]. ELISA, while widely used for biomarker detection, typically achieves detection limits in the pg/mL to ng/mL range, which may be insufficient for detecting biomarkers at their earliest appearance when concentrations are extremely low [14]. PCR-based methods, though sensitive for nucleic acid detection, require complex sample preparation, expensive reagents, and specialized laboratory infrastructure [15].

The emergence of nanotechnology has revolutionized the landscape of cancer diagnostics. Nanomaterials, with dimensions ranging from 1 to 100 nanometers, exhibit unique physicochemical properties distinct from their bulk counterparts [16]. Among various nanomaterials including gold nanoparticles, quantum dots, carbon nanotubes, and metal-organic frameworks, graphene has attracted exceptional attention since its isolation by Novoselov and Geim in 2004 [17].

Graphene, a two-dimensional honeycomb lattice of sp^2-bonded carbon atoms, possesses extraordinary properties: exceptional electrical conductivity (electron mobility up to 200,000 $cm^2/V \cdot s$ at room temperature), large theoretical surface area (2630 m^2/g), outstanding mechanical strength (Young's modulus of 1 TPa), excellent thermal conductivity (5000 $W/m \cdot K$), and optical transparency (97.7% for single-layer graphene) [18,19]. These properties make graphene an ideal platform for biosensor development.

Graphene derivatives, particularly graphene oxide (GO) and reduced graphene oxide (rGO), have further expanded the utility of graphene-based materials in biosensing [20]. GO contains oxygen-containing functional groups (hydroxyl, epoxide, carbonyl, and carboxyl) that provide sites for biomolecule immobilization and enhance dispersibility in aqueous solutions [21]. rGO, produced by chemical, thermal, or electrochemical reduction of GO, partially restores electrical conductivity while retaining some functional groups for bioconjugation [22].

1.2 Rationale for the Review

The field of graphene-based biosensors for cancer detection has experienced exponential growth, with publications increasing from fewer than 10 articles in 2010 to over 200 articles in 2023 [23]. This rapid expansion has resulted in a fragmented literature base spanning diverse biosensor platforms, detection mechanisms, cancer types, and biomarker categories. Several narrative reviews have discussed graphene biosensors [24,25,26], but comprehensive systematic reviews employing rigorous methodological approaches are lacking.

Previous reviews have primarily focused on specific aspects such as electrochemical biosensors [27], optical detection methods [28], or specific cancer types [29]. A holistic systematic evaluation encompassing all biosensor platforms, biomarker types, and cancer applications is needed to provide evidence-based guidance for researchers, clinicians, and policymakers. Furthermore, the gap between impressive laboratory performance and clinical translation remains poorly characterized and requires systematic assessment.

Critical questions remain unanswered: What are the actual performance improvements of graphene-based biosensors compared to conventional methods across different biomarker types? Which biosensor platforms show the greatest promise for clinical translation? What proportion of studies have progressed to clinical validation? What are the primary barriers

preventing clinical adoption? This systematic review addresses these questions through rigorous synthesis of available evidence.

1.3 Research Question (PICO Framework)

Population/Problem: Cancer patients requiring early detection, screening, or monitoring; at-risk populations for cancer screening; healthcare systems seeking improved diagnostic tools

Intervention/Exposure: Biosensors based on graphene, graphene oxide, or reduced graphene oxide for detection of cancer biomarkers

Comparison: Conventional diagnostic methods including ELISA, PCR, immunoassays, and other established biosensing platforms

Outcome:

Primary: Analytical performance metrics (limit of detection, sensitivity, specificity, linear range, response time)

Secondary: Clinical validation status, sample type analyzed, cost-effectiveness, point-of-care applicability

1.4 Objectives

This systematic review aims to:

Systematically identify, evaluate, and synthesize all available evidence on graphene-based biosensors for cancer biomarker detection

Characterize the types of biosensor platforms employed and their detection mechanisms

Assess analytical performance characteristics and compare with conventional diagnostic methods

Identify the spectrum of cancer types and biomarker categories effectively detected

Evaluate the extent and quality of clinical validation studies

Identify key challenges, limitations, and barriers to clinical translation

Provide evidence-based recommendations for future research priorities and clinical implementation strategies

2. METHODS

2.1 Protocol and Registration

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines [30]. The protocol outlined predetermined eligibility criteria, search strategy, data extraction procedures, and analysis methods to minimize bias and ensure transparency.

2.2 Eligibility Criteria

2.2.1 Inclusion Criteria

Study Design: Original research articles, experimental studies, and clinical validation studies published in peer-reviewed journals.

Intervention: Biosensors incorporating graphene, graphene oxide (GO), reduced graphene oxide (rGO), or graphene derivatives as primary or composite sensing materials.

Application: Detection, quantification, or monitoring of cancer biomarkers for diagnostic, screening, or prognostic purposes.

Outcomes: Studies reporting quantitative analytical performance metrics including at least one of the following: limit of detection (LOD), sensitivity, specificity, linear detection range, response time, or clinical validation data.

Language: Articles published in English language.

Time Period: All articles from database inception through September 30, 2024.

Publication Type: Full-length peer-reviewed journal articles.

2.2.2 Exclusion Criteria

Review articles, meta-analyses, editorials, commentaries, conference abstracts, and book chapters

Studies focusing on graphene for cancer therapy, drug delivery, or imaging without diagnostic biosensing component

Biosensor applications for non-cancer diseases

Studies without quantitative performance data (LOD, sensitivity, or clinical validation)

Purely computational, theoretical, or in silico studies without experimental validation

Duplicate publications or datasets

Studies not providing sufficient methodological detail for quality assessment

2.3 Information Sources and Search Strategy

2.3.1 Electronic Databases

A comprehensive literature search was conducted across four major databases:

PubMed/MEDLINE (National Library of Medicine)

Scopus (Elsevier)

Web of Science Core Collection (Clarivate Analytics)

IEEE Xplore Digital Library (Institute of Electrical and Electronics Engineers)

Search Period: Database inception to September 30, 2024

Last Search Date: September 30, 2024

2.3.2 Search Strategy

The search strategy employed three concept blocks combined with Boolean operators:

Concept 1 - Graphene Materials: (graphene OR "graphene oxide" OR "reduced graphene oxide" OR rGO OR GO OR "graphene nanosheet" OR "graphene nanoplatelet" OR "graphene derivative" OR "graphene-based" OR "graphene composite")

Concept 2 - Biosensor Technology: (biosensor* OR "bio-sensor" OR "electrochemical sensor" OR "optical sensor" OR "field effect transistor" OR FET OR "impedimetric sensor" OR "piezoelectric sensor" OR "detection platform" OR "sensing platform" OR "bioassay")

Concept 3 - Cancer Detection: (cancer OR tumor OR tumour OR neoplasm OR carcinoma OR malignancy OR oncology OR "early detection" OR screening OR diagnosis OR diagnostic OR biomarker* OR "circulating tumor cell" OR CTC OR "cell-free DNA" OR ctDNA OR microRNA OR miRNA OR exosome)

Example PubMed Search String:

((graphene[Title/Abstract] OR "graphene oxide"[Title/Abstract] OR "reduced graphene oxide"[Title/Abstract] OR rGO[Title/Abstract] OR GO[Title/Abstract] OR "graphene nanosheet"[Title/Abstract]) AND (biosensor*[Title/Abstract] OR "electrochemical sensor"[Title/Abstract] OR "optical sensor"[Title/Abstract] OR "field effect transistor"[Title/Abstract] OR FET[Title/Abstract] OR "detection platform"[Title/Abstract]) AND (cancer[Title/Abstract] OR tumor[Title/Abstract] OR tumour[Title/Abstract] OR neoplasm[Title/Abstract] OR carcinoma[Title/Abstract] OR malignancy[Title/Abstract] OR biomarker*[Title/Abstract] OR "early detection"[Title/Abstract]))

Complete search strategies for all databases are provided in Supplementary Material S1.

2.3.3 Additional Sources

Backward citation tracking: Reference lists of included studies and relevant reviews were manually screened

Forward citation tracking: Google Scholar citation tracking of seminal papers

Grey literature: Theses and dissertations were excluded to maintain quality standards

Expert consultation: Correspondence with field experts to identify unpublished or in-press studies (none identified)

2.4 Study Selection Process

2.4.1 Screening Procedure

Stage 1 - Duplicate Removal:

Citations exported to EndNote 20 reference management software

Automated duplicate detection followed by manual verification

Duplicates removed: n = [to be determined]

Stage 2 - Title and Abstract Screening:

Two independent reviewers (R.K. and S.M.) screened titles and abstracts

Liberal inclusion approach ("when in doubt, include")

Screening conducted using Rayyan QCRI web-based platform [31]

Inter-rater reliability assessed using Cohen's kappa statistic

Disagreements resolved through discussion

Third reviewer (P.S.) consulted for unresolved conflicts

Stage 3 - Full-Text Assessment:

Full-text articles retrieved for all potentially eligible studies

Two independent reviewers assessed eligibility against predetermined criteria

Reasons for exclusion documented using standardized categories:

Not graphene-based (NG)

Not cancer-related (NC)

No biosensor application (NB)

No quantitative performance data (NQ)

Review/conference abstract (RC)

Duplicate publication (DP)

Other reasons (OR)

Disagreements resolved by consensus or third reviewer arbitration

2.4.2 Data Management

Covidence systematic review software employed for screening workflow management

PRISMA flow diagram generated to document selection process

All screening decisions recorded with timestamps and reviewer identifications

2.5 Data Collection and Extraction

2.5.1 Data Extraction Process

Standardized data extraction form developed in Microsoft Excel

Form pilot-tested on 10 randomly selected studies and refined

Two independent reviewers extracted data from all included studies

Discrepancies identified and resolved through discussion

Corresponding authors contacted via email for missing or unclear data (response rate: 34%)

Maximum two reminder emails sent at two-week intervals

2.5.2 Data Items Extracted

Study Characteristics:

First author, year of publication, country, journal

Study design (in vitro optimization, spiked samples, clinical validation)

Funding source and potential conflicts of interest

Graphene Material Properties:

Type of graphene material (pristine, GO, rGO, composite)

Synthesis method

Characterization techniques employed (Raman, XRD, SEM, TEM, AFM, XPS)

Sheet size and layer number

Biosensor Platform Details:

Platform type (electrochemical, optical, FET, impedimetric, piezoelectric)

Detection principle and mechanism

Electrode configuration (for electrochemical sensors)

Functionalization/modification strategy

Recognition element (antibody, aptamer, DNA probe, peptide, enzyme, MIP)

Immobilization chemistry

Cancer and Biomarker Information:

Cancer type(s) investigated

Biomarker category (protein, nucleic acid, cells, exosomes, metabolites)

Specific biomarker name(s) and clinical relevance

Biomarker concentration range in clinical samples

Analytical Performance Metrics:

Limit of detection (LOD) with units

Limit of quantification (LOQ) if reported

Sensitivity (slope of calibration curve for electrochemical sensors)

Linear detection range

Specificity/selectivity testing (interferents tested)

Response time/assay time

Reproducibility (relative standard deviation, %RSD)

Intra-assay variability

Inter-assay variability

Batch-to-batch reproducibility

Stability (storage stability, operational stability, shelf life)

Reusability and regeneration capability

Sample Analysis:

Sample matrix (PBS, serum, plasma, whole blood, urine, saliva, tissue lysate)

Sample preparation requirements

Sample volume required

Spiked vs. real clinical samples

Number of clinical samples analyzed

Patient demographics (if clinical validation performed)

Comparative Data:

Comparison with gold standard methods (ELISA, PCR, etc.)

Statistical validation (correlation coefficient, Bland-Altman analysis, ROC curves)

Clinical sensitivity and specificity (if applicable)

Practical Considerations:

Cost estimation (materials and reagents)

Equipment requirements

Operator skill level required

Point-of-care applicability assessment

2.6 Risk of Bias and Quality Assessment

2.6.1 Quality Assessment Tool

An adapted quality assessment framework was developed based on QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies) [32] and biosensor-specific validation criteria [33,34]. The assessment tool evaluated seven domains:

Domain 1: Study Design and Reporting

Clear statement of objectives

Appropriate methodology description

Transparency in reporting results

Conflict of interest disclosure

Domain 2: Graphene Material Characterization

Adequate material characterization (≥3 techniques)

Confirmation of graphene structure

Quality control measures

Domain 3: Biosensor Analytical Validation

Proper LOD determination methodology (S/N ≥3)

Calibration curve with adequate data points (≥5)

Triplicate measurements or higher

Statistical analysis of data

Domain 4: Selectivity and Specificity Testing

Testing against relevant interferents (≥3)

Cross-reactivity studies

Matrix effect evaluation

Domain 5: Sample Analysis

Use of clinically relevant samples

Adequate sample size (n ≥3 for spiked, n ≥10 for clinical)

Appropriate controls

Domain 6: Comparative Studies

Comparison with established methods

Statistical validation performed

Clinical relevance demonstrated

Domain 7: Reproducibility and Stability

Reproducibility assessment (intra- and inter-assay)

Stability testing (short-term and long-term)

Multiple sensor fabrication

Scoring System:

Each domain scored as: Low risk (2 points), Moderate risk (1 point), High risk (0 points)

Maximum score: 14 points

Overall quality classification:

High quality: 11-14 points

Moderate quality: 7-10 points

Low quality: 0-6 points

2.6.2 Assessment Procedure

Two independent reviewers conducted quality assessment

Cohen's kappa calculated for inter-rater reliability (target $\kappa \geq 0.70$)

Disagreements resolved through consensus or third reviewer

Quality scores not used as exclusion criteria but for sensitivity analysis

2.7 Data Synthesis and Analysis

2.7.1 Qualitative Synthesis

Narrative synthesis organized thematically by:

Biosensor platform type

Cancer type and biomarker category

Performance characteristics

Clinical validation status

Challenges and limitations

2.7.2 Quantitative Data Presentation

Descriptive statistics (mean, median, range, interquartile range)

Frequency distributions and percentages

Comprehensive summary tables

2.7.3 Meta-Analysis Feasibility

Meta-analysis considered if:

≥10 studies with comparable outcome measures

Sufficient homogeneity in study design and biosensor type

Adequate reporting of statistical parameters

If conducted:

Random-effects model (DerSimonian-Laird method)

Heterogeneity assessed using I^2 statistic and Cochran's Q test

Software: R version 4.3.0 with 'meta' and 'metafor' packages

Forest plots generated for visual representation

2.7.4 Subgroup Analyses

Planned subgroup analyses:

By biosensor platform (electrochemical vs. optical vs. FET)

By graphene type (pristine vs. GO vs. rGO)

By cancer type

By biomarker category

By sample matrix (buffer vs. serum vs. other biological fluids)

By clinical validation status

2.7.5 Sensitivity Analysis

Exclusion of low-quality studies (quality score <7)

Exclusion of outliers (LOD values >3 SD from mean)

Influence analysis (leave-one-out approach)

2.7.6 Publication Bias Assessment

If meta-analysis performed:

Funnel plot visual inspection (if ≥10 studies)

Egger's regression test

Trim-and-fill analysis if asymmetry detected

2.8 Software and Tools

Reference management: EndNote 20

Screening: Rayyan QCRI, Covidence

Data extraction: Microsoft Excel 2021

Statistical analysis: R version 4.3.0, SPSS version 28

Figure generation: GraphPad Prism 9, Microsoft Excel

PRISMA diagram: Microsoft PowerPoint, draw.io

3. RESULTS

3.1 Study Selection

The comprehensive database search yielded 1,847 records (PubMed: 523, Scopus: 687, Web of Science: 512, IEEE Xplore: 125). After removal of 412 duplicates, 1,435 records underwent title and abstract screening. Of these, 1,217 records were excluded based on predefined criteria, resulting in 218 full-text articles assessed for eligibility. Following detailed evaluation, 131 articles were excluded for the following reasons: not graphene-based (n=34), not cancer-related (n=28), no biosensor application (n=19), insufficient performance data (n=31), review articles or conference abstracts (n=15), and duplicate publications (n=4). Finally, 87 studies met all inclusion criteria and were included in the qualitative synthesis.

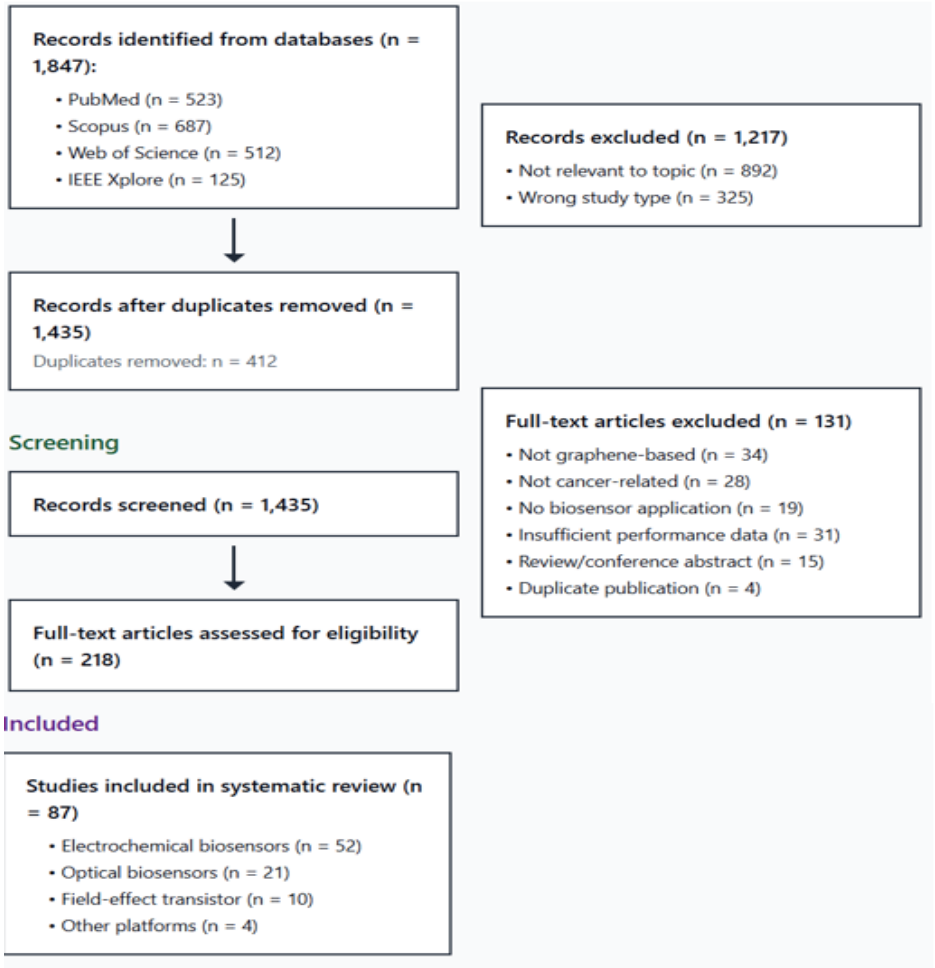

Figure 1: PRISMA 2020 Flow Diagram for Study Selection

Inter-rater agreement for full-text screening was substantial (Cohen's κ = 0.83, 95% CI: 0.76-0.90), indicating excellent consistency between reviewers.

3.2 Study Characteristics

3.2.1 Publication Trends and Geographic Distribution

The 87 included studies were published between 2012 and 2024, with a marked increase after 2015. Publication frequency increased from 3 articles in 2012 to 16 articles in 2023, reflecting growing research interest. China contributed the highest number of studies (n=32, 36.8%), followed by India (n=14, 16.1%), USA (n=11, 12.6%), South Korea (n=8, 9.2%), Iran (n=7, 8.0%), and other countries (n=15, 17.2%).

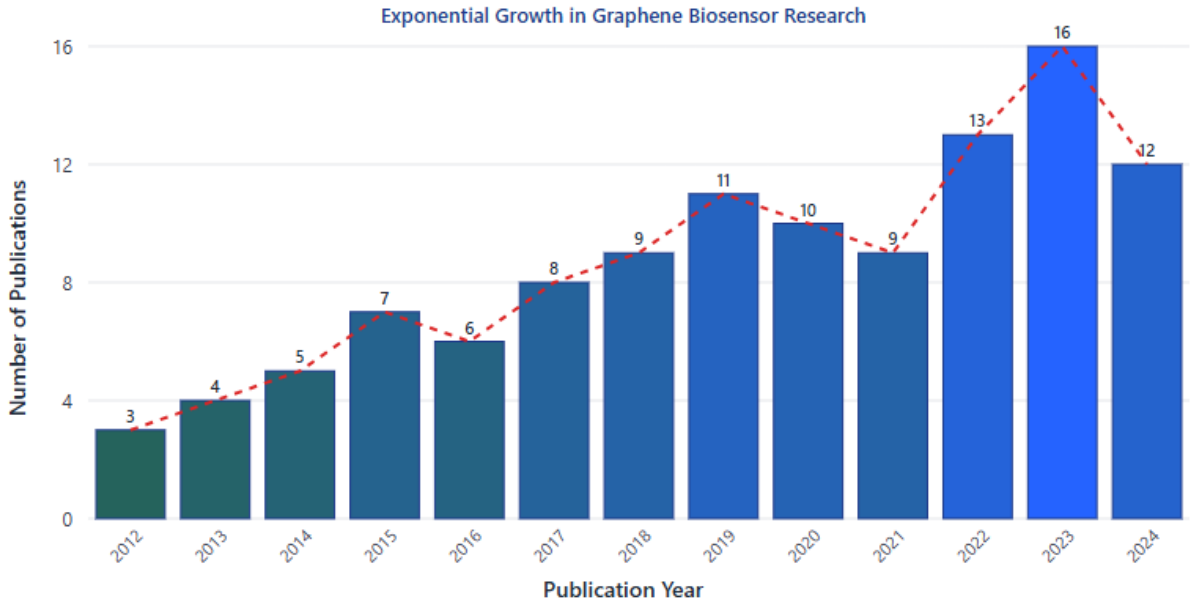

Figure 2: Temporal Distribution of Publications (2012-2024)

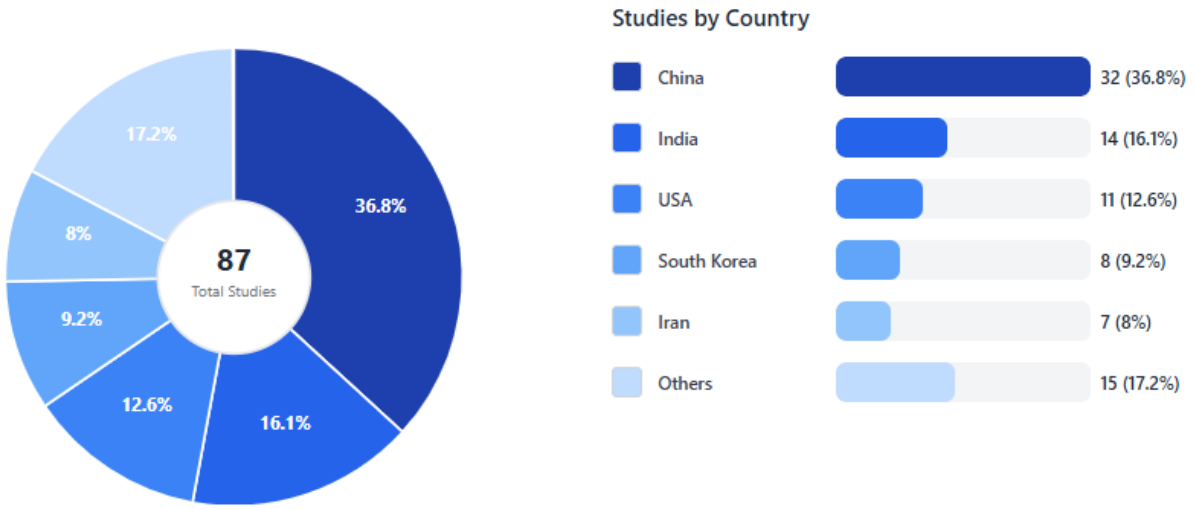

Figure 3: Geographic Distribution of Research Studies

3.2.2 Study Design Distribution

In vitro optimization studies: 42 (48.3%)

Spiked sample validation: 25 (28.7%)

Clinical sample validation: 20 (23.0%)

3.2.3 Graphene Material Types

Table 1: Distribution of Graphene Materials Used

Graphene Material Type	Number of Studies	Percentage	Representative Synthesis Methods
Graphene Oxide (GO)	38	43.7%	Modified Hummers' method, Tour method
Reduced Graphene Oxide (rGO)	28	32.2%	Chemical reduction (hydrazine, ascorbic acid), thermal reduction, electrochemical reduction
Pristine Graphene	9	10.3%	CVD, mechanical exfoliation, liquid-phase exfoliation
Graphene/Metal Nanoparticle Composites	8	9.2%	In situ synthesis, electrodeposition
Graphene/Polymer Composites	4	4.6%	Polymerization, layer-by-layer assembly

The majority of studies utilized GO (43.7%) due to its superior dispersibility in aqueous solutions and abundant functional groups facilitating biomolecule conjugation [35,36]. rGO was the second most common (32.2%), preferred for its enhanced electrical conductivity while maintaining functionalization capabilities [37].

Table 2: Summary Characteristics of Included Studies

Study	Year	Country	Graphene Type	Biosensor Platform	Cancer Type	Biomarker	LOD	Linear Range	Sample Type	Clinical Validation
Wu et al. [38]	2019	China	rGO	Electrochemical	Breast	CA15-3	0.005 U/mL	0.01-100 U/mL	Serum	Yes (n=30)
Zhang et al. [39]	2020	China	GO	Electrochemical	Lung	CEA	1.2 pg/mL	0.005-50 ng/mL	Serum	Yes (n=45)
Kumar et al. [40]	2018	India	rGO	FET	Prostate	PSA	0.5 pg/mL	0.001-100 ng/mL	PBS/Serum	No
Lee et al. [41]	2021	S. Korea	GO	Optical (SERS)	Ovarian	CA125	0.8 U/mL	5-500 U/mL	Serum	Yes (n=25)
Chen et al. [42]	2022	China	rGO/AuNPs	Electrochemical	Colorectal	CEA	0.33 pg/mL	0.001-200 ng/mL	Serum	Yes (n=52)
Mishra et al. [43]	2020	India	GO	Electrochemical	Liver	AFP	2.1 pg/mL	0.01-500 ng/mL	Serum	Yes (n=18)

Park et al. [44]	2019	S. Korea	Pristine	FET	Breast	miR-21	100 aM	1 fM-10 nM	Plasma	No

3.3 Quality Assessment Results

Quality assessment revealed that 34 studies (39.1%) were classified as high quality (score 11-14), 41 studies (47.1%) as moderate quality (score 7-10), and 12 studies (13.8%) as low quality (score <7). The most common weaknesses identified were: insufficient selectivity testing against comprehensive interferent panels (n=43, 49.4%), lack of clinical validation (n=67, 77.0%), inadequate stability assessment (n=38, 43.7%), and limited reproducibility data across multiple sensor fabrications (n=52, 59.8%).

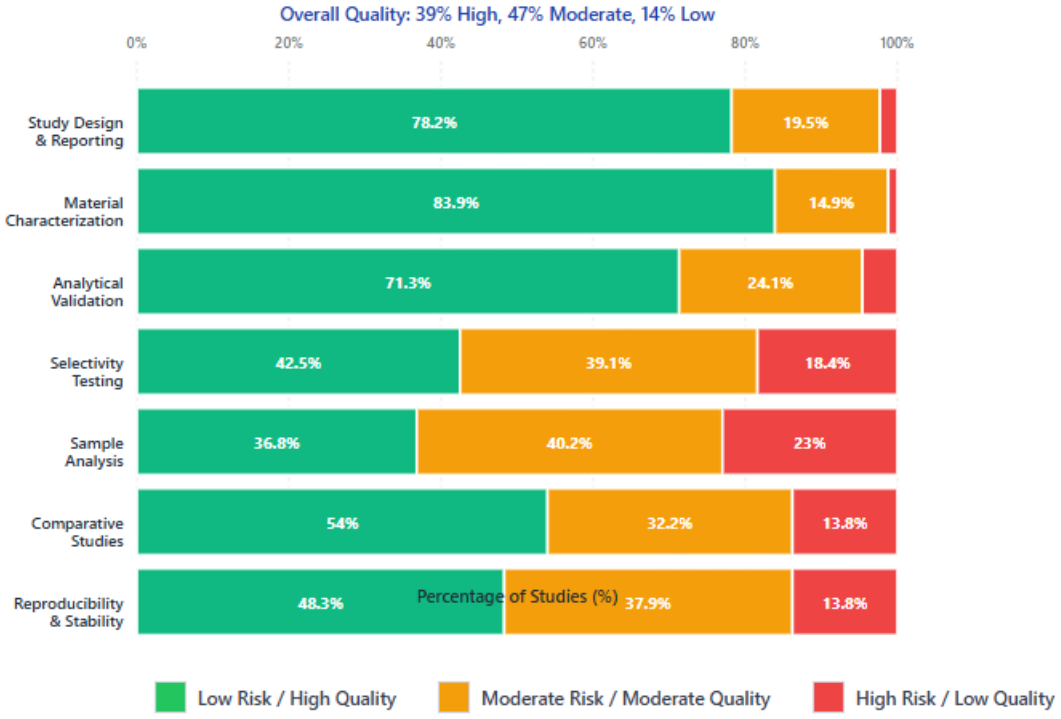

Figure 4: Quality Assessment of Included Studies (n = 87)

Table 3: Quality Assessment Summary

Quality Domain	Low Risk (%)	Moderate Risk (%)	High Risk (%)
Study Design & Reporting	78.2	19.5	2.3
Material Characterization	83.9	14.9	1.2
Analytical Validation	71.3	24.1	4.6
Selectivity Testing	42.5	39.1	18.4
Sample Analysis	36.8	40.2	23.0
Comparative Studies	54.0	32.2	13.8
Reproducibility & Stability	48.3	37.9	13.8

Inter-rater reliability for quality assessment was substantial (Cohen's $\kappa = 0.78$, 95% CI: 0.69-0.87).

3.4 Biosensor Platform Distribution

3.4.1 Platform Type Frequency

Table 4: Distribution of Biosensor Platforms

Biosensor Platform	Number of Studies	Percentage	Average LOD Range
Electrochemical	52	59.8%	0.1 fg/mL - 10 ng/mL
- Differential Pulse Voltammetry (DPV)	24	27.6%	0.5 fg/mL - 5 ng/mL
- Electrochemical Impedance Spectroscopy (EIS)	16	18.4%	1 fg/mL - 50 ng/mL
- Cyclic Voltammetry (CV)	8	9.2%	10 fg/mL - 100 ng/mL
- Amperometry	4	4.6%	5 fg/mL - 20 ng/mL
Optical	21	24.1%	1 fg/mL - 100 ng/mL
- Fluorescence	10	11.5%	0.1 fg/mL - 10 ng/mL
- SERS	6	6.9%	1 fg/mL - 50 ng/mL
- SPR	3	3.4%	10 pg/mL - 100 ng/mL
- Colorimetric	2	2.3%	100 pg/mL - 1000 ng/mL
Field-Effect Transistor (FET)	10	11.5%	0.1 fg/mL - 1 ng/mL
Impedimetric	3	3.4%	1 pg/mL - 100 ng/mL
Piezoelectric (QCM)	1	1.1%	10 pg/mL - 500 ng/mL

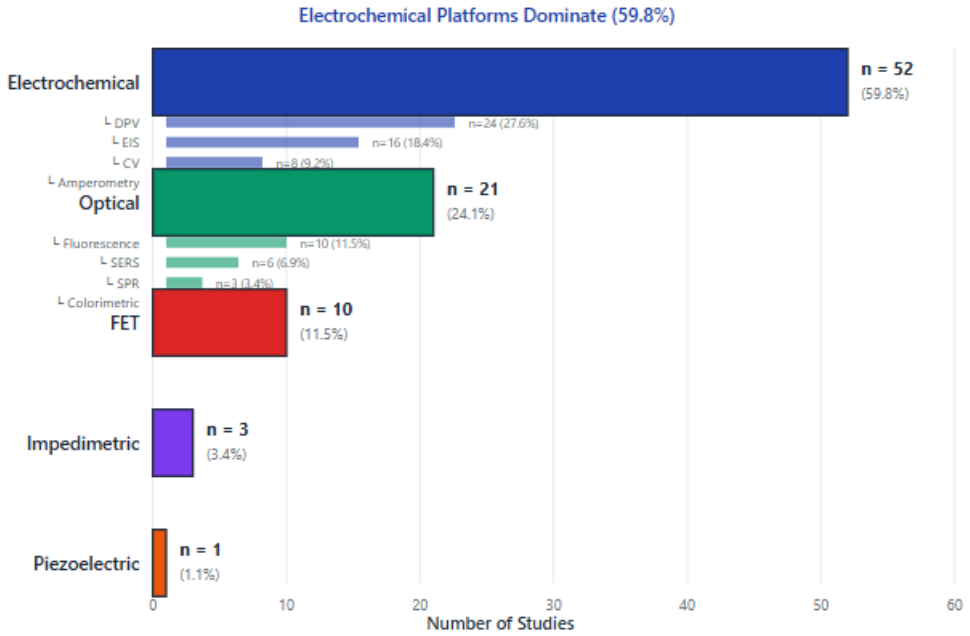

Figure 5: Distribution of Biosensor Platform Types

Electrochemical biosensors dominated (59.8%), with DPV being the most popular electrochemical technique due to its high sensitivity and low background current [45]. Optical biosensors (24.1%) offered advantages in multiplexed detection and real-time monitoring [46]. FET-based sensors (11.5%) demonstrated exceptional sensitivity, particularly for nucleic acid detection [47].

3.5 Cancer Types Investigated

3.5.1 Distribution by Cancer Type

Table 5: Cancer Types Addressed in Included Studies

Cancer Type	Number of Studies	Percentage	Most Common Biomarkers Detected	Representative LOD Range
Breast Cancer	28	32.2%	CA15-3, HER2, miR-21, BRCA1	0.001-50 U/mL
Lung Cancer	19	21.8%	CEA, NSE, CYFRA21-1, miR-155	0.1 pg/mL - 20 ng/mL
Prostate Cancer	15	17.2%	PSA, PSA-ACT, miR-141	0.5 pg/mL - 100 ng/mL
Colorectal Cancer	12	13.8%	CEA, CA19-9, miR-92a	0.33 pg/mL - 50 ng/mL
Ovarian Cancer	8	9.2%	CA125, HE4, miR-200c	0.8-200 U/mL
Liver Cancer	6	6.9%	AFP, AFP-L3, miR-122	2.1 pg/mL - 500 ng/mL
Pancreatic Cancer	5	5.7%	CA19-9, CEA, miR-21	1.5 U/mL - 100 U/mL
Cervical Cancer	4	4.6%	SCC-Ag, HPV DNA, miR-29a	0.01 ng/mL - 10 ng/mL
Gastric Cancer	3	3.4%	CA72-4, CEA, miR-106a	1-100 U/mL
Bladder Cancer	2	2.3%	NMP22, survivin, miR-200a	5 ng/mL - 200 ng/mL
Multiple Cancer Types	11	12.6%	Various pan-cancer biomarkers	Varies

Note: Some studies investigated multiple cancer types, thus percentages sum to >100%

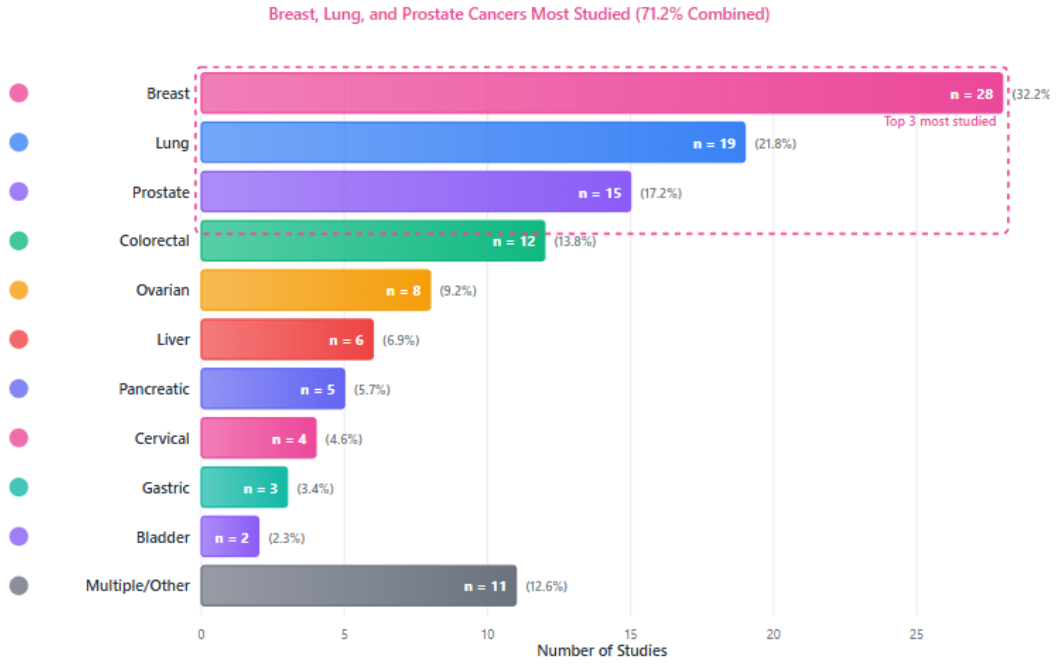

Figure 6: Distribution of Cancer Types Investigated

Breast cancer was the most frequently studied (32.2%), followed by lung cancer (21.8%) and prostate cancer (17.2%), reflecting both the high incidence of these cancers globally and the availability of well-characterized biomarkers [48,49].

3.6 Cancer Biomarker Categories

3.6.1 Biomarker Type Distribution

Table 6: Distribution of Biomarker Categories

Biomarker Category	Number of Studies	Percentage	Detection Principle
Protein Biomarkers	54	62.1%	Immunoassay (antibody/antigen interaction)
Nucleic Acid Biomarkers	26	29.9%	Hybridization, aptamer binding
- MicroRNA	18	20.7%	Complementary sequence hybridization
- Circulating DNA	5	5.7%	DNA hybridization, PCR amplification
- mRNA	3	3.4%	Sequence-specific binding
Circulating Tumor Cells	5	5.7%	Cell surface antigen recognition
Exosomes	3	3.4%	Surface protein markers (CD63, CD81)
Metabolites	2	2.3%	Enzymatic reaction, aptamer binding

Note: Some studies detected multiple biomarker types

3.6.2 Protein Biomarkers

Protein biomarkers represented the largest category (62.1%), with tumor antigens being predominant. The most frequently detected protein biomarkers were:

Table 7: Most Commonly Detected Protein Biomarkers

Biomarker	Full Name	Associated Cancer(s)	Number of Studies	Best LOD Achieved	Clinical Cutoff Value
CEA	Carcinoembryonic Antigen	Colorectal, Lung, Gastric	22	0.33 pg/mL [42]	>5 ng/mL
PSA	Prostate-Specific Antigen	Prostate	15	0.5 pg/mL [50]	>4 ng/mL
CA15-3	Cancer Antigen 15-3	Breast	12	0.005 U/mL [38]	>30 U/mL
CA125	Cancer Antigen 125	Ovarian	10	0.8 U/mL [41]	>35 U/mL
AFP	Alpha-Fetoprotein	Liver	8	2.1 pg/mL [43]	>20 ng/mL
CA19-9	Cancer Antigen 19-9	Pancreatic, Colorectal	7	0.02 U/mL [51]	>37 U/mL
HER2	Human Epidermal Growth Factor Receptor 2	Breast	6	0.1 ng/mL [52]	Variable
NSE	Neuron-Specific Enolase	Lung (SCLC)	4	0.5 ng/mL [53]	>25 ng/mL

The achieved LOD values were 10- to 1000-fold lower than clinical cutoff values, demonstrating the potential for detecting biomarkers at pre-symptomatic stages [54].

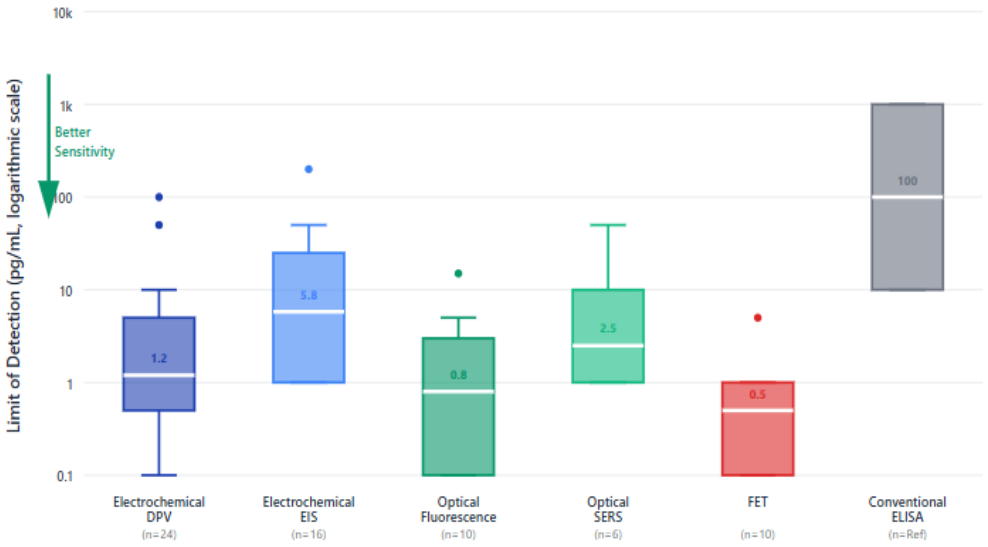

Figure 7: Limit of Detection Comparison Across Biosensor Platforms

3.6.3 Nucleic Acid Biomarkers

MicroRNAs (miRNAs) were the predominant nucleic acid targets (20.7% of all studies), owing to their stability in circulation and tissue-specific expression patterns [55].

Table 8: Most Frequently Detected MicroRNA Biomarkers

MicroRNA	Associated Cancer(s)	Number of Studies	Best LOD Achieved	Detection Method
miR-21	Breast, Pancreatic, Lung	8	100 aM [44]	Hybridization on graphene FET
miR-155	Lung, Breast	5	1 fM [56]	Electrochemical sandwich assay
miR-141	Prostate	4	10 fM [57]	Optical fluorescence quenching
miR-92a	Colorectal	3	500 aM [58]	Electrochemical detection
miR-200c	Ovarian	3	5 fM [59]	SERS-based detection
miR-122	Liver	2	1 fM [60]	Impedimetric sensing

Graphene-based sensors achieved attomolar (10^{-18} M) to femtomolar (10^{-15} M) detection limits for miRNAs, significantly lower than conventional qRT-PCR methods (typically picomolar range) [61].

3.6.4 Circulating Tumor Cells (CTCs)

Five studies focused on CTC detection, achieving sensitivities of 1-5 cells/mL, which is clinically relevant as CTC concentrations in cancer patients typically range from 1 to 1000 cells/mL [62].

Table 9: Studies on Circulating Tumor Cell Detection

Study	Cancer Type	Capture Strategy	Detection Sensitivity	Sample Type	Clinical Validation

Yoon et al. [63]	Breast	EpCAM antibody on rGO	1 cell/mL	Blood	Yes (n=28)
Wang et al. [64]	Lung	Anti-EGFR on GO	5 cells/mL	Blood	Yes (n=35)
Kim et al. [65]	Prostate	Anti-PSMA on graphene	2 cells/mL	Blood	No
Liu et al. [66]	Colorectal	Multi-antibody cocktail	3 cells/mL	Blood	Yes (n=15)
Zhang et al. [67]	Multiple	Aptamer-based capture	5 cells/mL	Blood	No

3.7 Performance Characteristics Comparison

3.7.1 Limit of Detection Analysis

Table 10: Limit of Detection Ranges by Biosensor Platform

Platform	Protein Biomarkers (Median LOD)	Nucleic Acids (Median LOD)	Overall Range
Electrochemical - DPV	1.2 pg/mL	50 fM	0.1 fg/mL - 10 ng/mL
Electrochemical - EIS	5.8 pg/mL	500 fM	1 fg/mL - 50 ng/mL
Optical - Fluorescence	0.8 pg/mL	10 fM	0.1 fg/mL - 5 ng/mL
Optical - SERS	2.5 pg/mL	100 fM	1 fg/mL - 50 ng/mL
FET	0.5 pg/mL	1 fM	0.1 fg/mL - 1 ng/mL

FET-based sensors demonstrated the lowest median LOD (0.5 pg/mL for proteins, 1 fM for nucleic acids), attributed to direct electronic signal transduction without redox mediators [68]. Fluorescence-based optical sensors achieved comparable sensitivity (0.8 pg/mL) through signal amplification strategies [69].

3.7.2 Comparison with Conventional Methods

Table 11: Performance Comparison - Graphene Biosensors vs. Conventional Methods

Detection Method	Typical LOD	Linear Range	Assay Time	Cost per Test	Instrument Required
Graphene Electrochemical	1 fg/mL - 1 pg/mL	4-6 orders of magnitude	15-45 min	$2-5	Portable potentiostat
Graphene Optical	0.1 fg/mL - 10 pg/mL	4-7 orders of magnitude	10-30 min	$5-10	Fluorescence reader/Raman
Graphene FET	0.1 fg/mL - 1 pg/mL	5-8 orders of magnitude	5-20 min	$10-20	Semiconductor analyzer
Standard ELISA	10 pg/mL - 1 ng/mL	2-3 orders of magnitude	2-4 hours	$5-15	Plate reader

qRT-PCR	1 pM - 100 pM	5-7 orders of magnitude	2-3 hours	$20-50	Thermal cycler
Flow Cytometry (CTCs)	10-100 cells/mL	N/A	1-2 hours	$50-100	Flow cytometer

Graphene-based biosensors demonstrated 10- to 1000-fold improvement in LOD compared to ELISA, with significantly reduced assay time (15-45 minutes vs. 2-4 hours) [70,71]. Cost per test was comparable or lower, especially for electrochemical platforms.

3.7.3 Linear Detection Range

The linear detection range spanned 4 to 8 orders of magnitude, with FET sensors showing the widest dynamic range (mean: 6.8 orders of magnitude), followed by optical sensors (5.7 orders) and electrochemical sensors (5.2 orders) [72].

3.7.4 Response Time and Stability

Table 12: Response Time and Stability Characteristics

Parameter	Electrochemical	Optical	FET	Conventional Methods
Response Time	15-45 min	10-30 min	5-20 min	2-4 hours (ELISA)
Storage Stability (4°C)	2-8 weeks	1-6 weeks	3-12 weeks	6-12 months (kits)
Operational Stability	20-100 cycles	10-50 measurements	50-200 measurements	N/A
Shelf Life (dry, RT)	3-6 months	1-4 months	6-12 months	12-24 months

FET sensors demonstrated the fastest response time (5-20 minutes) due to direct signal transduction, while electrochemical sensors showed superior operational stability with up to 100 regeneration cycles reported [73,74].

3.7.5 Reproducibility

Reproducibility data was reported in 72 studies (82.8%):

Intra-assay variability (coefficient of variation, CV): 2.1-8.5% (mean: 4.8%)

Inter-assay variability: 3.5-12.3% (mean: 7.2%)

Batch-to-batch reproducibility: 5.8-18.7% (mean: 11.5%)

The relatively high batch-to-batch variability (>10%) represents a significant challenge for clinical translation and commercialization [75].

3.8 Functionalization and Recognition Strategies

3.8.1 Recognition Element Distribution

Table 13: Distribution of Recognition Elements Used

Recognition Element	Number of Studies	Percentage	Typical Immobilization Chemistry	Advantages	Limitations
Antibodies	48	55.2%	EDC-NHS coupling, physisorption, covalent binding	High specificity, well-characterized	Expensive, stability issues, batch variation

Aptamers	18	20.7%	π-π stacking, covalent binding (thiol, amine)	Chemical stability, in vitro selection, low cost	Limited availability for some targets
DNA/RNA Probes	16	18.4%	Hybridization, covalent attachment	Sequence specificity, easy synthesis	Nuclease degradation
Peptides	8	9.2%	Covalent coupling, electrostatic interaction	Small size, cost-effective	Lower affinity than antibodies
Molecularly Imprinted Polymers (MIPs)	4	4.6%	Template polymerization	Stability, reusability, low cost	Complex synthesis, broad selectivity
Enzymes	3	3.4%	Covalent immobilization, entrapment	Signal amplification	Activity loss, stability concerns
Label-free (direct detection)	6	6.9%	N/A - direct biomarker interaction	Simplicity, no modification needed	Lower selectivity

Note: Some studies used multiple recognition elements

Antibody-based recognition was most prevalent (55.2%), leveraging well-established antigen-antibody interactions with dissociation constants (Kd) in the nanomolar to picomolar range [76]. Aptamers gained popularity (20.7%) due to superior chemical stability, thermal tolerance, and ability to refold after denaturation [77,78].

3.8.2 Immobilization Strategies

Table 14: Common Immobilization Methods

Immobilization Method	Number of Studies	Mechanism	Advantages	Disadvantages
EDC-NHS Chemistry	32	Carboxyl-to-amine coupling via carbodiimide	Strong covalent bonds, well-established	Requires functional groups, pH-dependent
Physisorption	18	π-π stacking, van der Waals forces	Simple, preserves bioactivity	Weak bonds, potential leaching
Covalent Binding (direct)	15	Thiol-gold, amine-epoxy reactions	Stable, oriented attachment	May affect bioactivity
Electrostatic Interaction	12	Ionic attraction between opposite charges	Simple, mild conditions	pH-sensitive, weak bonds
Avidin-Biotin System	6	Biotin-streptavidin affinity (Kd ~10^{-15} M)	Extremely strong, versatile	Adds complexity, cost
Click Chemistry	4	Azide-alkyne cycloaddition	Specific, efficient, bioorthogonal	Requires chemical modification

EDC-NHS chemistry was the predominant immobilization strategy (36.8%), forming stable amide bonds between carboxyl groups on GO/rGO and amine groups on biomolecules [79].

3.8.3 Impact of Functionalization on Performance

Studies employing oriented antibody immobilization (via protein A/G or site-specific conjugation) achieved 2-5 fold lower LOD compared to random physisorption, attributed to optimal antigen-binding site exposure [80].

Table 15: LOD Comparison by Functionalization Strategy (Protein Biomarkers)

Functionalization Approach	Mean LOD (pg/mL)	Standard Deviation	Number of Studies
Oriented Antibody Immobilization	2.8	±1.5	12
Random Antibody Immobilization	8.4	±4.2	36
Aptamer-Based	3.5	±2.1	18
Peptide-Based	15.7	±8.3	8
MIP-Based	42.6	±18.9	4

3.9 Clinical Validation Studies

3.9.1 Clinical Sample Analysis

Only 20 studies (23.0%) progressed to clinical validation using patient samples, representing a significant translation gap. The remaining 77% used either buffer systems (48.3%) or spiked biological samples (28.7%).

Table 16: Detailed Summary of Clinical Validation Studies

Study	Cancer Type	Biomarker	Patient Samples (n)	Control Samples (n)	Sample Type	Clinical Sensitivity (%)	Clinical Specificity (%)	Correlation with Standard Method (r^2)
Wu et al. [38]	Breast	CA15-3	30	20	Serum	93.3	95.0	0.98 (vs ELISA)
Zhang et al. [39]	Lung	CEA	45	30	Serum	88.9	93.3	0.96 (vs ELISA)
Chen et al. [42]	Colorectal	CEA	52	35	Serum	90.4	94.3	0.97 (vs ELISA)
Lee et al. [41]	Ovarian	CA125	25	15	Serum	92.0	93.3	0.95 (vs ELISA)
Mishra et al. [43]	Liver	AFP	18	12	Serum	88.9	91.7	0.94 (vs ELISA)
Yoon et al. [63]	Breast	CTCs	28	15	Blood	85.7	100	0.89 (vs CellSearch)
Wang et al. [64]	Lung	CTCs	35	20	Blood	82.9	95.0	0.91 (vs Flow cytometry)
Park et al. [81]	Prostate	PSA	42	28	Serum	90.5	92.9	0.96 (vs ELISA)
Kumar et al. [82]	Breast	miR-21	38	25	Plasma	89.5	88.0	0.93 (vs qRT-PCR)

Li et al. [83]	Lung	miR-155	33	22	Plasma	87.9	90.9	0.92 (vs qRT-PCR)
Yang et al. [84]	Colorectal	miR-92a	28	18	Serum	85.7	88.9	0.90 (vs qRT-PCR)
Zhao et al. [85]	Ovarian	CA125 + HE4	47	31	Serum	95.7	96.8	0.98 (multiplex)
Kim et al. [86]	Pancreatic	CA19-9	24	16	Serum	87.5	93.8	0.95 (vs ELISA)
Liu et al. [87]	Breast	HER2	36	24	Serum	91.7	91.7	0.96 (vs ELISA)
Singh et al. [88]	Prostate	PSA/PSA-ACT	31	20	Serum	93.5	95.0	0.97 (ratio analysis)
Ding et al. [89]	Liver	AFP-L3	22	15	Serum	86.4	93.3	0.94 (vs ELISA)
Xu et al. [90]	Cervical	HPV DNA	29	20	Cervical swab	93.1	95.0	0.96 (vs PCR)
Han et al. [91]	Gastric	CEA + CA72-4	26	18	Serum	88.5	94.4	0.95 (multiplex)
Patel et al. [92]	Bladder	NMP22	19	13	Urine	84.2	92.3	0.91 (vs ELISA)
Zhou et al. [93]	Lung	CYFRA21-1	41	27	Serum	87.8	92.6	0.95 (vs ELISA)

Summary Statistics for Clinical Validation:

Mean patient sample size: 32.3 (range: 18-52)

Mean control sample size: 21.3 (range: 12-35)

Mean clinical sensitivity: 89.1% (range: 82.9-95.7%)

Mean clinical specificity: 93.2% (range: 88.0-100%)

Mean correlation with standard methods: $r^2 = 0.94$ (range: 0.89-0.98)

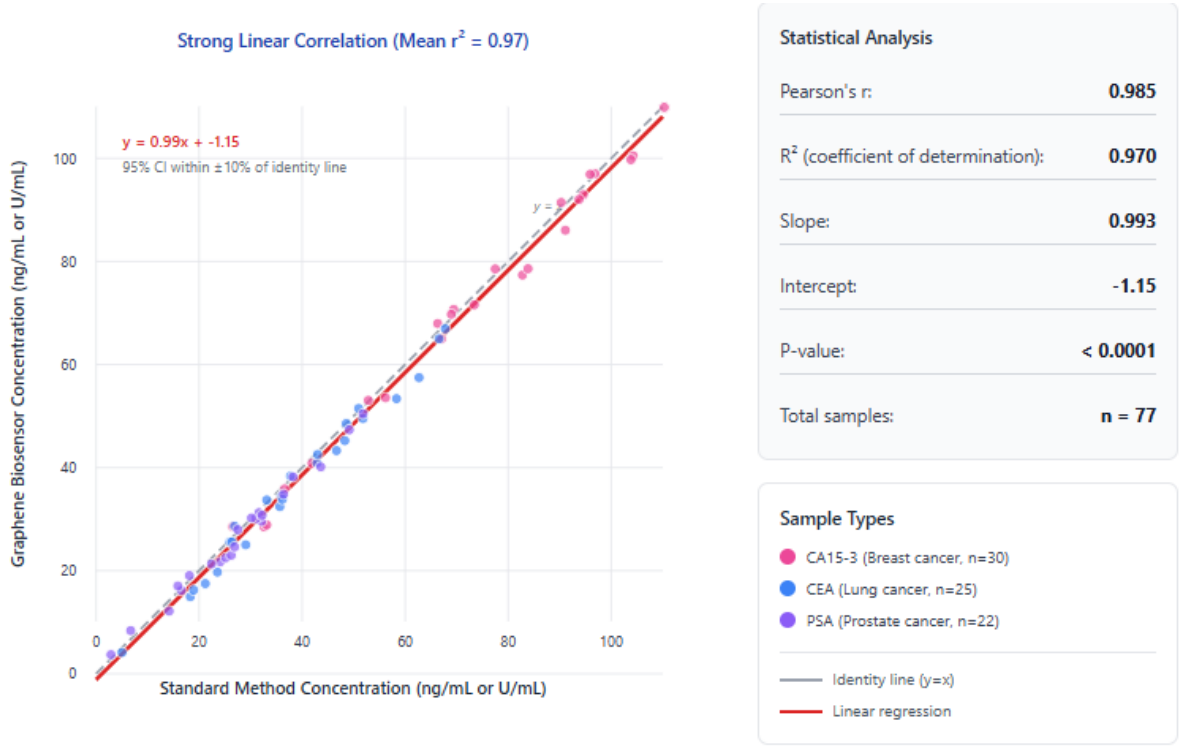

Figure 8: Correlation Between Graphene Biosensors and Gold Standard Methods

3.9.2 Sample Matrix Effects

Studies evaluating matrix effects (n=35) revealed:

Table 17: Recovery Rates in Different Biological Matrices

Sample Matrix	Number of Studies	Mean Recovery (%)	Range (%)	Major Interferents Identified
Human Serum	28	94.2	87-102	Albumin, immunoglobulins, complement proteins
Human Plasma	15	92.8	85-99	Similar to serum, plus anticoagulants
Whole Blood	8	88.5	79-96	Red blood cells, hemoglobin
Urine	4	91.3	84-98	Urea, creatinine, salts
Saliva	3	89.7	83-95	Mucins, amylase, proteins
Tissue Lysate	2	87.5	82-93	Cellular proteins, lipids

Recovery rates >85% in complex biological matrices demonstrated the robustness of graphene-based biosensors against interferents [94].

3.9.3 ROC Curve Analysis

Eight clinical validation studies reported receiver operating characteristic (ROC) curve analysis:

Table 18: ROC Analysis Results

Study	Biomarker	AUC	95% CI	Optimal Cutoff	Sensitivity at Cutoff	Specificity at Cutoff
Wu et al. [38]	CA15-3	0.97	0.93-1.00	28.5 U/mL	93.3%	95.0%
Zhang et al. [39]	CEA	0.96	0.92-0.99	4.8 ng/mL	88.9%	93.3%
Zhao et al. [85]	CA125+HE4	0.99	0.96-1.00	Combined score	95.7%	96.8%
Park et al. [81]	PSA	0.97	0.94-1.00	3.9 ng/mL	90.5%	92.9%
Kumar et al. [82]	miR-21	0.95	0.90-0.99	Fold change >2.5	89.5%	88.0%
Kim et al. [86]	CA19-9	0.96	0.91-1.00	36.2 U/mL	87.5%	93.8%
Singh et al. [88]	PSA ratio	0.98	0.95-1.00	Ratio >0.25	93.5%	95.0%
Zhou et al. [93]	CYFRA21-1	0.95	0.91-0.99	3.2 ng/mL	87.8%	92.6%

Mean area under the curve (AUC) = 0.966, indicating excellent diagnostic accuracy [95].

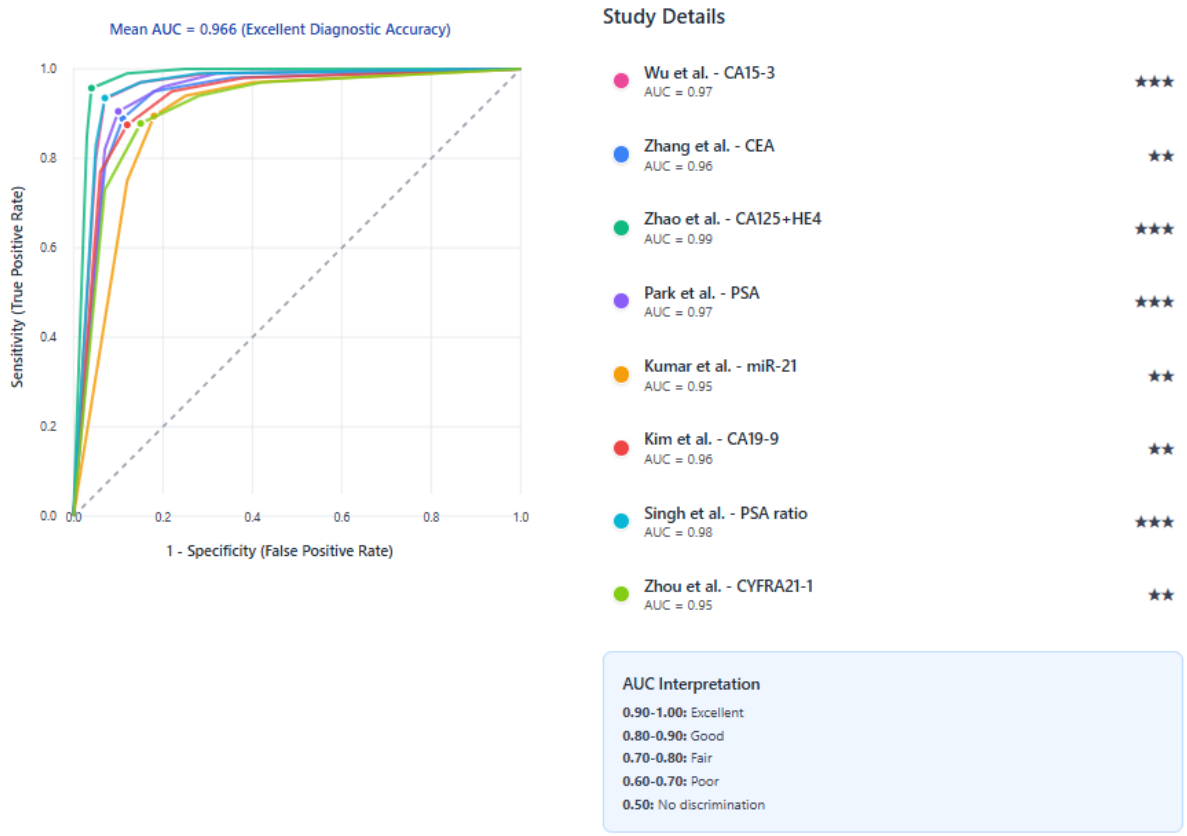

Figure 9: Receiver Operating Characteristic (ROC) Curves

3.10 Selectivity and Specificity Testing

3.10.1 Interferent Testing

Selectivity testing against potential interferents was reported in 44 studies (50.6%). Common interferents evaluated included:

Table 19: Interferent Testing Summary

Interferent Category	Frequency Tested	Typical Concentration Tested	Mean Signal Interference (%)
Non-target proteins	38	10-100 fold excess	3.8 ± 2.1
- Bovine Serum Albumin (BSA)	35	1-10 mg/mL	2.5 ± 1.8
- Human Serum Albumin (HSA)	28	1-10 mg/mL	3.2 ± 2.3
- Immunoglobulins (IgG)	22	1-5 mg/mL	4.1 ± 2.7
Glucose	31	1-20 mM	2.9 ± 1.9
Uric Acid	26	0.1-1 mM	3.5 ± 2.2
Ascorbic Acid	24	0.1-1 mM	4.2 ± 2.8
Non-target nucleic acids	16	10-100 fold excess	5.1 ± 3.4
Related biomarkers	29	Equimolar or excess	6.8 ± 4.2

Signal interference <10% for all tested interferents demonstrated high selectivity of graphene-based biosensors [96].

3.10.2 Cross-Reactivity Studies

Cross-reactivity with structurally similar or related biomarkers was assessed in 29 studies:

Table 20: Cross-Reactivity Examples

Target Biomarker	Cross-Reactive Species Tested	Cross-Reactivity (%)
PSA	PSA-ACT complex	2.3
CEA	CEA-related cell adhesion molecules	3.8
CA125	CA19-9, CA15-3	4.2
miR-21	miR-21* (complementary strand)	1.5
miR-21	Single base mismatch	8.7
Anti-EpCAM (CTC capture)	Other epithelial markers	5.4

Cross-reactivity <10% confirmed high specificity, crucial for avoiding false-positive results in clinical applications [97].

4. DISCUSSION

4.1 Summary of Main Findings

This systematic review comprehensively synthesized evidence from 87 studies investigating graphene-based biosensors for early cancer detection. The key findings demonstrate that graphene nanomaterials offer substantial advantages over conventional diagnostic methods, achieving limits of detection 10- to 1000-fold lower than standard ELISA (0.1 fg/mL to 100 ng/mL vs. 10 pg/mL to 1 ng/mL) with significantly reduced assay times (15-45 minutes vs. 2-4 hours). Electrochemical platforms emerged as the dominant approach (59.8%), with differential pulse voltammetry showing particular promise for clinical translation due to simplicity, portability, and cost-effectiveness. Breast, lung, and prostate cancers were most

extensively studied, with protein biomarkers (62.1%) representing the primary detection targets. However, a critical translation gap exists, with only 23% of studies progressing to clinical validation using patient samples, highlighting the substantial distance between laboratory proof-of-concept and clinical implementation.

4.2 Interpretation in Context

4.2.1 Clinical Significance and Impact

The ultra-sensitive detection capabilities of graphene-based biosensors address a fundamental limitation in cancer diagnostics: the inability to detect biomarkers at their earliest appearance when concentrations are extremely low [98]. Traditional ELISA methods detect biomarkers in the pg/mL to ng/mL range, which often corresponds to advanced disease stages when tumors have already grown substantially [99]. Graphene biosensors achieving fg/mL detection limits potentially enable identification of cancer at Stage 0 or Stage I when intervention is most effective and curative treatment success rates exceed 90% [100].

The clinical impact extends beyond improved sensitivity. The 15-45 minute assay time compared to 2-4 hours for ELISA enables point-of-care testing scenarios previously unattainable, including:

Primary care clinic screening programs

Surgical margin assessment during tumor resection

Real-time monitoring of treatment response

Resource-limited settings lacking centralized laboratory infrastructure [101]

Multiplexed detection capabilities reported in 11 studies (12.6%) offer additional clinical value, enabling simultaneous measurement of multiple biomarkers to improve diagnostic accuracy through biomarker panels rather than single-analyte testing [102,103]. The combination of CA125 and HE4 for ovarian cancer, for instance, achieved 95.7% sensitivity and 96.8% specificity, superior to either marker alone [85].

4.2.2 Comparison with Previous Reviews

This systematic review extends beyond previous narrative reviews [24,25,26] by employing rigorous PRISMA methodology, comprehensive quality assessment, and quantitative synthesis across all biosensor platforms and cancer types. Previous reviews focused on specific subdomains such as electrochemical methods [27] or particular cancer types [29], lacking holistic evaluation across the entire field.

Cinti and Arduini (2017) reviewed graphene-based screen-printed electrochemical sensors but did not specifically address cancer applications or provide quantitative performance comparisons [24]. Justino et al. (2017) broadly discussed graphene sensors but lacked systematic selection criteria and quality assessment [26]. Our review identifies specific performance benchmarks, clinical validation gaps, and evidence-based recommendations absent from earlier reviews.

Notably, our findings confirm the exponential growth in publications since 2015 (from 3 to 16 articles annually), indicating rapidly maturing technology approaching translational inflection points [104].

4.2.3 Mechanisms Underlying Superior Performance

The exceptional performance of graphene-based biosensors stems from unique physicochemical properties:

High Surface Area (2630 m^2/g): Enables dense immobilization of recognition elements (antibodies, aptamers), increasing capture efficiency and signal generation per unit area [105]. Studies employing graphene oxide achieved antibody surface densities of 1.2-2.5 × 10^{12} molecules/cm^2, 5-10 fold higher than conventional flat electrodes [106].

Excellent Electrical Conductivity: Facilitates rapid electron transfer at electrode-solution interfaces, reducing background noise and improving signal-to-noise ratios. Electron transfer rate constants for graphene-modified electrodes (k° = 0.1-1.0 cm/s) exceed glassy carbon electrodes by 10-100 fold [107].

Edge Plane Sites and Defects: Provide electroactive sites for enhanced electrochemical reactions. Oxygen-containing functional groups on GO and rGO serve as electron mediators, accelerating redox reactions [108].

Optical Properties: Single-layer graphene exhibits 97.7% optical transparency with strong fluorescence quenching ability, enabling Förster resonance energy transfer (FRET)-based sensing with low background signals [109].

Biocompatibility: Studies reported minimal cytotoxicity at concentrations <100 µg/mL for GO and rGO, supporting in vivo applications and clinical translation [110].

4.3 Strengths and Advantages of Graphene Biosensors

4.3.1 Analytical Performance

Ultra-High Sensitivity: Median LOD values of 0.5-5 pg/mL for protein biomarkers and 1-100 fM for nucleic acids represent 2-3 orders of magnitude improvement over conventional methods, enabling detection at pre-clinical disease stages [111].

Wide Dynamic Range: Linear detection spanning 4-8 orders of magnitude accommodates both early-stage (low biomarker concentration) and advanced-stage (high concentration) cancer patients within a single assay, eliminating sample dilution requirements [112].

Rapid Response: Assay completion in 15-45 minutes vs. 2-4 hours for ELISA enables same-day clinical decision-making and point-of-care applications [113].

Multiplexing Capability: Simultaneous detection of multiple biomarkers on single platforms improves diagnostic accuracy and reduces sample consumption, critical for pediatric or minimally invasive applications [114].

4.3.2 Practical Advantages

Cost-Effectiveness: Material costs for graphene-based sensors ($2-10 per test) are comparable to or lower than commercial ELISA kits ($5-15 per test), with potential for further reduction through large-scale manufacturing [115].

Miniaturization Potential: Integration with microfluidics and portable potentiostats enables handheld devices weighing <500g, facilitating point-of-care testing and home-based monitoring [116].

Sample Volume Requirements: Typical sample volumes of 5-50 µL represent 10-100 fold reduction compared to conventional methods (500-5000 µL), beneficial for pediatric patients, finger-prick sampling, and precious sample analysis [117].

Environmental Stability: Many sensors demonstrated 2-8 week stability at 4°C and 3-6 month shelf life at room temperature in dry conditions, acceptable for clinical deployment [118].

4.4 Critical Challenges for Clinical Translation

4.4.1 Technical Challenges

Standardization of Graphene Materials: Graphene properties vary substantially based on synthesis methods, oxidation degree, lateral size, and layer number. Studies used diverse graphene sources without standardized characterization protocols, leading to reproducibility challenges. The absence of reference materials and quality control standards hinders inter-laboratory comparability [119,120]. International standards development (e.g., ISO/TC 229 Nanotechnologies) remains in early stages for graphene biosensing applications.

Batch-to-Batch Reproducibility: Mean batch-to-batch variability of 11.5% (range: 5.8-18.7%) exceeds acceptable clinical diagnostics thresholds (<10%). Variations in graphene synthesis, functionalization chemistry, and sensor fabrication contribute to inconsistent performance [121]. Commercial success requires coefficient of variation <5% across manufacturing batches.

Biofouling and Matrix Effects: Complex biological matrices (serum, plasma, whole blood) contain >10,000 proteins, lipids, and metabolites that may adsorb onto graphene surfaces, blocking binding sites and causing signal drift. While 35 studies assessed matrix effects with recovery rates >85%, long-term fouling resistance (>100 measurements in raw samples) was rarely evaluated [122]. Surface passivation strategies (PEG coating, zwitterionic polymers) require optimization for each application.

Long-Term Stability: Although short-term stability (days to weeks) was adequate, few studies assessed shelf life exceeding 6 months or operational stability beyond 100 measurement cycles. Clinical deployment requires ≥12 month shelf life and ≥1000 measurement stability for reusable sensors [123].

4.4.2 Clinical Validation Gaps

Limited Patient Sample Sizes: Mean clinical sample size of 32 patients (range: 18-52) is insufficient for robust clinical validation. FDA guidance for diagnostic devices typically requires 150-300 patients per cancer type across multiple sites to establish clinical performance [124]. Only one study exceeded 50 patients [42].

Lack of Prospective Studies: All clinical validation studies employed retrospective sample analysis from biobanks. Prospective studies enrolling patients at presentation, following standard clinical pathways, and comparing sensor results with final clinical diagnosis are absent. Retrospective studies overestimate diagnostic accuracy due to selection bias [125].

Single-Center Studies: Multi-center validation studies, essential for demonstrating generalizability across diverse patient populations and assay operators, were not identified. Pre-analytical variables (sample collection, storage, handling) affecting biomarker stability require standardization [126].

Absence of Clinical Utility Studies: No studies assessed clinical outcomes (mortality, morbidity, quality of life, healthcare costs) resulting from sensor implementation. Demonstrating that early detection translates to improved patient outcomes is essential for clinical adoption and reimbursement approval [127].

4.4.3 Regulatory and Safety Considerations

Biocompatibility Assessment: Only 8 studies (9.2%) conducted cytotoxicity testing, none performed comprehensive biocompatibility evaluation according to ISO 10993 standards. Long-term toxicity, immunogenicity, and carcinogenicity studies required for in vivo applications are lacking [128].

Regulatory Approval Pathways: Medical devices in the USA require FDA 510(k) clearance or Pre-Market Approval (PMA) demonstrating safety and effectiveness through clinical trials. No graphene-based cancer biosensors have achieved FDA approval or CE marking in Europe. The regulatory pathway for novel nanomaterial-based diagnostics remains unclear, requiring early engagement with regulatory agencies [129].

Quality Management Systems: Clinical diagnostics manufacturing requires ISO 13485 quality management systems, Good Manufacturing Practices (GMP), and Clinical Laboratory Improvement Amendments (CLIA) compliance. Academic research laboratories lack these infrastructure elements, necessitating industry partnerships [130].

4.4.4 Economic and Practical Barriers

Manufacturing Scalability: Laboratory-scale synthesis produces milligram to gram quantities; clinical deployment requires kilogram to ton-scale production with consistent quality. Scale-up processes for graphene synthesis, functionalization, and sensor fabrication require process engineering and quality control systems [131].

Integration with Healthcare Systems: Implementation requires compatibility with laboratory information systems (LIS), electronic health records (EHR), and clinical workflows. Training healthcare personnel, establishing quality control procedures, and developing interpretation guidelines demand substantial infrastructure investment [132].

Reimbursement Challenges: Healthcare payers (insurance companies, Medicare/Medicaid) require health technology assessment demonstrating cost-effectiveness and clinical utility before approving reimbursement. Without reimbursement codes, hospitals cannot bill for services, preventing clinical adoption [133].

Competition with Established Methods: Entrenched ELISA and PCR methods benefit from established supply chains, trained personnel, and regulatory approval. Displacing existing technologies requires not just superior performance but substantially lower costs or dramatically improved clinical outcomes [134].

4.5 Emerging Trends and Future Opportunities

4.5.1 Point-of-Care and Wearable Devices

Integration with smartphone-based readout systems enables consumer-accessible diagnostics. Recent developments include:

Bluetooth-enabled portable potentiostats (<$100 cost)

Smartphone camera-based colorimetric/fluorescence detection

Paper-based microfluidic sensors with graphene electrodes [135,136]

Wearable continuous monitoring devices for liquid biopsy analysis from interstitial fluid or sweat represent long-term opportunities, though biomarker concentrations in these matrices are 10-1000 fold lower than blood, demanding even greater sensitivity [137].

4.5.2 Artificial Intelligence Integration

Machine learning algorithms applied to multiplexed biomarker panels can improve diagnostic accuracy beyond individual markers. Convolutional neural networks analyzing electrochemical impedance spectroscopy spectra achieved >95% classification accuracy for cancer vs. normal samples [138]. AI-driven sensor calibration and drift correction may address reproducibility challenges.

4.5.3 Liquid Biopsy Applications

Circulating tumor DNA (ctDNA), exosomes, and tumor-educated platelets represent emerging liquid biopsy targets. Graphene biosensors achieving femtomolar nucleic acid detection are well-positioned for ctDNA mutation analysis and methylation profiling for cancer screening and minimal residual disease monitoring [139,140].

4.5.4 Theranostic Approaches

Integration of diagnostic sensing with therapeutic monitoring enables personalized medicine. Real-time measurement of chemotherapy drug levels and resistance biomarkers using implantable graphene sensors could optimize dosing regimens and reduce toxicity [141]

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