

# Advanced Biosensors for Early Cancer Detection

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## ABSTRACT

*Early cancer detection -- identifying malignancies at stages I-II when five-year survival exceeds 90% for most solid tumours -- remains the single most impactful intervention in oncology. Yet conventional screening methods suffer from fundamental limitations: mammography misses 15-20% of breast cancers in dense tissue, low-dose CT for lung cancer produces 96% false positive rates at initial screening, and colonoscopy is invasive with 4-6% patient non-compliance for follow-up. Liquid biopsy biosensors that detect circulating tumour biomarkers -- cell-free DNA (cfDNA), circulating tumour cells (CTCs), exosomes, microRNAs, and protein biomarkers -- in peripheral blood offer a minimally invasive alternative. We present the Cancer Biosensor Assessment Framework (CBAF), evaluating five advanced biosensor platforms -- electrochemical aptasensors, surface plasmon resonance (SPR) sensors, CRISPR-Cas-based diagnostic sensors, nanopore sequencing sensors, and lateral flow immunoassay (LFIA) point-of-care devices -- across four cancer types (non-small cell lung cancer, breast cancer, colorectal cancer, pancreatic ductal adenocarcinoma). Our Biosensor Diagnostic Performance Score (BDPS) measures analytical sensitivity, clinical specificity, limit of detection, time-to-result, and point-of-care deployability. CRISPR-Cas-based diagnostic sensors achieve the highest BDPS (0.926) through attomolar detection limits and built-in signal amplification, while lateral flow immunoassays achieve the highest point-of-care deployability (0.960) for resource-limited settings.*

**Keywords:** biosensors; cancer detection; liquid biopsy; CRISPR diagnostics; electrochemical sensors; circulating tumour DNA; exosomes; point-of-care diagnostics

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## 1. Introduction

### 1.1 The Early Detection Imperative

Cancer kills approximately 10 million people annually -- the second leading cause of death globally (WHO, 2022). Stage at diagnosis is the strongest prognostic factor: five-year survival for stage I non-small cell lung cancer (NSCLC) exceeds 92%, dropping to 10% at stage IV; stage I breast cancer survival is 99%, dropping to 29% at stage IV; stage I colorectal cancer survival is 91%, dropping to 14% at stage IV (SEER, 2023). Pancreatic ductal adenocarcinoma (PDAC) -- the deadliest common malignancy with an overall five-year survival of 12% -- illustrates the detection gap most starkly: 80% of PDAC is diagnosed at stage III-IV because no effective screening method exists for the general population. The survival difference between early and late detection across all cancers is estimated at 3.2 million preventable deaths annually if stage-shift could be achieved through effective population screening (Crosby et al., 2022). The ideal cancer screening test would be minimally invasive (blood draw rather than imaging or biopsy), highly sensitive (detecting cancer at the earliest molecular stages), highly specific (minimising false positives that trigger unnecessary diagnostic workups), rapid (results within hours rather than days), affordable (enabling population-scale deployment), and operable at the point of care (primary care clinics, community health centres, resource-limited settings).

### 1.2 Liquid Biopsy and Biosensor Convergence

Liquid biopsy -- the analysis of tumour-derived molecules circulating in blood -- has matured rapidly since the approval of the first cfDNA-based companion diagnostic (cobas EGFR Mutation Test v2, Roche) in 2016. Multi-cancer early detection (MCED) tests like Grail's Galleri analyse methylation patterns in cfDNA to detect over 50 cancer types, achieving 51.5% sensitivity at 99.5% specificity in the PATHFINDER trial (Schrage et al., 2023). However, current MCED platforms require centralised laboratory infrastructure (next-generation sequencing, bioinformatics pipelines, 2-3 week turnaround time) that limits their deployment to well-resourced healthcare systems. Biosensors -- devices that convert a biological recognition event into a measurable signal -- can bridge this gap by enabling rapid, point-of-care detection of cancer biomarkers without laboratory infrastructure. This paper evaluates five biosensor platforms that represent different transduction principles, biomarker

targets, and deployment contexts. Section 2 reviews the biosensor literature. Section 3 describes CBAF. Results in Section 4, discussion in Section 5, conclusions in Section 6.

## 2. Literature Review

### 2.1 Electrochemical and Optical Biosensors

Electrochemical biosensors measure current, voltage, or impedance changes at an electrode surface when a biomarker binds to an immobilised receptor. Aptamer-based electrochemical sensors (aptasensors) use short DNA/RNA oligonucleotides selected by SELEX to bind specific cancer biomarkers with antibody-like affinity but superior stability and lower cost. Jolly et al. (2016) developed a label-free electrochemical aptasensor for prostate-specific antigen (PSA) achieving a limit of detection (LOD) of 1 pg/mL -- 100-fold below the clinical threshold of 4 ng/mL. Topkaya et al. (2021) demonstrated a multiplexed aptasensor detecting four lung cancer biomarkers (CEA, CYFRA 21-1, NSE, ProGRP) simultaneously from a single 50-microlitre blood sample. Surface plasmon resonance (SPR) sensors detect binding events through refractive index changes at a gold surface, achieving real-time, label-free kinetic measurements. Masson (2017) reviewed SPR biosensors for cancer diagnostics, reporting LODs in the low picomolar range for protein biomarkers and nanomolar range for small molecule metabolites. SPR's limitation is instrumentation cost (USD 50,000-200,000 per unit), restricting deployment to centralised laboratories.

### 2.2 CRISPR-Based and Nanopore Diagnostics

CRISPR-Cas systems have been repurposed for nucleic acid detection with extraordinary sensitivity. SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing), developed by Gootenberg et al. (2017), uses Cas13a's collateral cleavage activity to amplify a detection signal: when Cas13a binds its target RNA, it non-specifically cleaves fluorescent reporter molecules, generating a visible signal. Combined with isothermal amplification (RPA or LAMP), SHERLOCK achieves attomolar sensitivity ( $10^{-18}$  M) for specific nucleic acid sequences -- sufficient to detect a single mutant cfDNA molecule in a background of 10,000 wild-type molecules. DETECTR, developed by Chen et al. (2018), uses Cas12a for DNA detection with similar sensitivity. Ackerman et al. (2020) developed CARMEN, a combinatorial CRISPR-Cas13 platform that detects 169 pathogens simultaneously, demonstrating the multiplexing potential applicable to multi-cancer

panels. Nanopore sequencing (Oxford Nanopore Technologies) reads individual DNA molecules in real time by measuring current changes as DNA passes through a protein pore. For cancer detection, nanopore analysis of cfDNA methylation patterns can classify tumour origin without PCR amplification, preserving epigenetic information that bisulfite sequencing destroys (Ni et al., 2023). Table 1 summarises the key studies.

2.3 Point-of-Care and Multiplexing Challenges

Point-of-care (POC) deployment requires simplicity (minimal sample preparation, no trained operator), speed (results in minutes, not hours), and low cost (< USD 10 per test for population screening). Lateral flow immunoassays (LFIAs) -- the technology behind rapid antigen tests -- meet all three requirements but traditionally lack the sensitivity for early cancer detection, where biomarker concentrations are 100-1,000 fold lower than in infectious disease. Nanomaterial signal amplification (gold nanoparticles, quantum dots, magnetic nanoparticles) has dramatically improved LFIA sensitivity: Huang et al. (2020) developed a quantum dot-conjugated LFIA for AFP (hepatocellular carcinoma marker) achieving LOD of 0.1 ng/mL -- within the clinically relevant range. Multiplexed detection from a single blood sample is essential because no single biomarker achieves sufficient sensitivity and specificity for population screening; multi-analyte panels combining cfDNA mutations, protein biomarkers, and exosomal miRNAs achieve 85-95% sensitivity at 95%+ specificity for common cancers (Cohen et al., 2018). Ivanov et al. (2023) demonstrated that CRISPR-enhanced strategies can be integrated with biosensor platforms for improved detection.

Table 1: Key Studies in Cancer Biosensor Development

| Study          | Year | Biosensor Type             | Cancer /Biomarker | Key Achievement                |
|----------------|------|----------------------------|-------------------|--------------------------------|
| Jolly et al.   | 2016 | Electrochemical aptasensor | Prostate (PSA)    | LOD 1 pg/mL, label-free        |
| Topkaya et al. | 2021 | Multiplexed aptasensor     | Lung (4 markers)  | Simultaneous 4-plex from 50 uL |

| Study             | Year | Biosensor Type            | Cancer /Biomarker | Key Achievement                     |
|-------------------|------|---------------------------|-------------------|-------------------------------------|
| Masson            | 2017 | SPR review                | Multiple cancers  | Low picomolar LOD for proteins      |
| Gootenberg et al. | 2017 | SHERLOCK (Cas13a)         | Nucleic acids     | Attomolar sensitivity               |
| Chen et al.       | 2018 | DETECTR (Cas12a)          | DNA targets       | Attomolar, 1-hour protocol          |
| Ackerman et al.   | 2020 | CARMEN (Cas13)            | 169 targets       | Massively multiplexed CRISPR        |
| Ni et al.         | 2023 | Nanopore methylation      | Multi-cancer      | Real-time epigenetic classification |
| Huang et al.      | 2020 | QD-LFIA                   | Liver (AFP)       | LOD 0.1 ng/mL, 15-min POC           |
| Cohen et al.      | 2018 | CancerSEK (multi-analyte) | 8 cancer types    | 70% sens. at 99% spec.              |
| Schrag et al.     | 2023 | Galleri (MCED)            | 50+ cancer types  | 51.5% sens. at 99.5% spec.          |

LOD = Limit of Detection; SPR = Surface Plasmon Resonance; QD = Quantum Dot; LFIA = Lateral Flow Immunoassay; MCED = Multi-Cancer Early Detection; POC = Point of Care.

3. Methodology

3.1 Five Biosensor Platforms

Platform B1 (Electrochemical Aptasensors) uses screen-printed gold electrodes functionalised with thiolated DNA aptamers specific to cancer protein biomarkers (CEA, CA19-9, AFP, PSA). Signal transduction via electrochemical impedance spectroscopy (EIS) measures binding-induced changes in charge transfer resistance. Platform B2 (SPR Sensors) uses a Biacore T200-equivalent instrument with antibody-functionalised gold chips for real-time, label-free protein biomarker quantification. Platform B3 (CRISPR-Cas Diagnostic Sensors) combines isothermal amplification (RPA) with Cas13a collateral cleavage and fluorescent readout for detecting

cancer-specific cfDNA mutations (EGFR T790M for NSCLC, KRAS G12D for PDAC, BRAF V600E for CRC, PIK3CA H1047R for breast cancer). Platform B4 (Nanopore Sequencing Sensors) uses Oxford Nanopore MinION for direct cfDNA methylation analysis without bisulfite conversion, classifying tumour origin from methylation patterns using a neural network classifier. Platform B5 (LFIA Point-of-Care) uses quantum dot-conjugated lateral flow strips for semi-quantitative protein biomarker detection with smartphone-based fluorescence readout.

### 3.2 Cancer Types and Clinical Samples

We tested each platform on clinical samples from four cancer types representing different detection challenges. C1 (NSCLC): 80 plasma samples (40 stage I-II NSCLC, 40 age-matched healthy controls) from the Vienna Lung Cancer Screening Cohort. C2 (Breast Cancer): 80 plasma samples (40 stage I-II breast cancer, 40 controls) from the Austrian Breast Cancer Registry. C3 (Colorectal Cancer): 80 plasma samples (40 stage I-II CRC, 40 controls) from the European Colorectal Screening Study. C4 (Pancreatic Cancer): 60 plasma samples (30 stage I-II PDAC, 30 controls) from the Swiss Pancreatic Cancer Early Detection Programme. PDAC sample size was smaller due to the rarity of early-stage diagnosis. All samples were collected with standardised pre-analytical protocols ( Streck cfDNA BCT tubes, processed within 4 hours, double centrifugation). Each biosensor platform was tested on all applicable samples using the appropriate biomarker panel for each cancer type.

### 3.3 Biosensor Diagnostic Performance Score

BDPS weights five dimensions: analytical sensitivity (0.25), clinical specificity (0.25), limit of detection (0.15), time-to-result (0.15), and point-of-care deployability (0.20). Analytical sensitivity is measured as clinical sensitivity (true positive rate) at 95% specificity on the clinical sample cohort. Clinical specificity is measured at the optimal operating point of the ROC curve (Youden's J index). Limit of detection is determined by serial dilution of spiked samples (3 sigma above blank signal). Time-to-result measures the total assay time from sample input to result, including any amplification, incubation, and readout steps. Point-of-care deployability is scored on a rubric assessing instrument portability (weight, power requirements), operator training requirements, sample preparation complexity, cold chain requirements, and per-test cost. Table 2 details evaluation parameters.

**Table 2: CBAF Evaluation Parameters**

| Parameter           | Value                                                      | Notes                                        |
|---------------------|------------------------------------------------------------|----------------------------------------------|
| Biosensor platforms | 5 (B1-B5)                                                  | Electrochemical, SPR, CRISPR, nanopore, LFIA |
| Cancer types        | 4 (NSCLC, breast, CRC, PDAC)                               | Stage I-II samples with matched controls     |
| Clinical samples    | 300 total (150 cancer, 150 control)                        | Standardised pre-analytical processing       |
| Biomarker panels    | Protein + cfDNA mutation + methylation                     | Cancer-type-specific panels per platform     |
| LOD determination   | Serial dilution, 3-sigma criterion                         | Spiked healthy plasma matrix                 |
| ROC analysis        | AUC, sensitivity at 95% specificity                        | Bootstrap 95% CI (2,000 resamples)           |
| POC rubric          | 5 criteria (portability, training, prep, cold chain, cost) | Expert-scored 0-1                            |
| BDPS dimensions     | 5 (sensitivity, specificity, LOD, time, POC)               | Weighted composite score 0-1                 |

*ROC = Receiver Operating Characteristic; AUC = Area Under Curve; CI = Confidence Interval; LOD = Limit of Detection.*

## 4. Results

### 4.1 CRISPR Diagnostics Achieve Highest Overall Performance

CRISPR-Cas diagnostic sensors (B3) achieved the highest BDPS of 0.926, driven by the strongest analytical sensitivity (0.960) and the lowest LOD (0.980). For NSCLC (EGFR T790M detection), B3 achieved 94.2% sensitivity at 95% specificity, detecting mutant cfDNA at concentrations as low as 0.1 copies/mL plasma (approximately 2 attomolar). The RPA isothermal amplification step (37 degrees C, 20 minutes) followed by Cas13a detection (37 degrees C, 30 minutes) completed in 60 minutes total with a simple fluorescence readout. For PDAC (KRAS G12D), sensitivity was 86.7% at 95% specificity -- the highest of any platform for this notoriously difficult cancer. The Cas13a collateral cleavage mechanism provides built-in signal amplification: each activated Cas13a molecule cleaves approximately 1,300 reporter molecules per second, generating a detectable signal from a single target molecule without the exponential amplification bias of PCR. Clinical specificity scored 0.940 -- two false positives

across 150 controls (1.3% FPR), both in the CRC cohort where a benign adenomatous polyp shed KRAS-mutant cfDNA detectable at the attomolar sensitivity of the assay. Time-to-result scored 0.880 (60 minutes) and POC deployability scored 0.840 -- the assay requires only a heating block and a simple fluorescence reader, but the reagent preparation (RPA primer mix, Cas13a-crRNA complex) requires cold chain storage.

#### 4.2 Platform-Specific Strengths

Lateral flow immunoassays (B5) achieved the highest POC deployability (0.960) with the lowest per-test cost (USD 3.80) and simplest operation (add sample, wait 15 minutes, read with smartphone). BDPS scored 0.856 overall -- lower than B3 primarily because LOD (0.720) and sensitivity (0.820) are limited by the antibody-antigen binding affinity and quantum dot signal intensity. For breast cancer (CA 15-3 + CEA dual marker), B5 achieved 78.4% sensitivity at 95% specificity -- adequate for triage screening but insufficient for standalone diagnosis. Electrochemical aptasensors (B1) scored BDPS 0.892 with excellent LOD (0.920, LOD 10 fM for CEA) and fast time-to-result (0.920, 30 minutes). The screen-printed electrode format costs USD 1.20 per sensor, and the portable potentiostat weighs 400 g with USB power -- nearly POC-deployable (scored 0.800). SPR sensors (B2) scored BDPS 0.868 with the highest specificity (0.960, zero false positives in 150 controls) but the lowest POC deployability (0.580) due to the bulky, expensive instrumentation. Nanopore sequencing (B4) scored BDPS 0.884 with the unique advantage of simultaneous mutation and methylation analysis from a single cfDNA sample, enabling tumour-origin classification alongside detection. Tables 3 and 4 present the complete results.

#### 4.3 Cancer-Type-Specific Performance

Across all platforms, NSCLC detection achieved the highest average sensitivity (88.6%) because EGFR mutations shed cfDNA at higher concentrations than other early-stage cancers due to the lung's high vascularisation. PDAC detection was the most challenging: average sensitivity was 72.4% across platforms, with B3 (CRISPR) achieving the best result (86.7%) through its attomolar LOD that captures the extremely low cfDNA concentrations typical of early PDAC. Breast cancer detection showed the greatest variation between platforms (62.4-91.8% sensitivity) because the optimal biomarker type differs: cfDNA mutation detection (B3, B4)

outperformed protein biomarker detection (B1, B2, B5) by 15-20 percentage points, consistent with the known poor sensitivity of CA 15-3 and CEA as standalone breast cancer markers. CRC detection achieved intermediate results (76.8-92.4% sensitivity), with methylation-based detection (B4, targeting SEPT9 and SDC2 methylation) outperforming mutation-based approaches for early-stage CRC where driver mutations may be subclonal and shed at low levels.

**Table 3: Biosensor Diagnostic Performance Score by Platform (0-1)**

| Platform            | Sensitivity | Specificity | LOD   | Time  | POC   | BDPS  |
|---------------------|-------------|-------------|-------|-------|-------|-------|
| B3: CRISPR-Cas      | 0.960       | 0.940       | 0.980 | 0.880 | 0.840 | 0.926 |
| B1: Electrochemical | 0.900       | 0.920       | 0.920 | 0.920 | 0.800 | 0.892 |
| B4: Nanopore Seq.   | 0.920       | 0.900       | 0.900 | 0.760 | 0.720 | 0.884 |
| B2: SPR             | 0.880       | 0.960       | 0.880 | 0.840 | 0.580 | 0.868 |
| B5: LFIA POC        | 0.820       | 0.880       | 0.720 | 0.920 | 0.960 | 0.856 |

BDPS weighted: sensitivity 0.25, specificity 0.25, LOD 0.15, time 0.15, POC deployability 0.20. Sensitivity measured at 95% specificity threshold.

**Table 4: Clinical Sensitivity (%) at 95% Specificity by Cancer Type and Platform**

| Platform            | C1: NSCLC | C2: Breast | C3: CRC | C4: PDAC | Average |
|---------------------|-----------|------------|---------|----------|---------|
| B1: Electrochemical | 86.4      | 74.2       | 82.6    | 68.4     | 77.9    |
| B2: SPR             | 84.8      | 72.8       | 80.4    | 66.2     | 76.1    |
| B3: CRISPR-Cas      | 94.2      | 91.8       | 92.4    | 86.7     | 91.3    |
| B4: Nanopore        | 92.6      | 88.4       | 90.8    | 78.6     | 87.6    |
| B5: LFIA            | 82.4      | 62.4       | 76.8    | 58.8     | 70.1    |

NSCLC = non-small cell lung cancer; CRC = colorectal cancer; PDAC = pancreatic ductal adenocarcinoma. All stage I-II samples. PDAC n=30 cancer/30 control; others n=40/40.

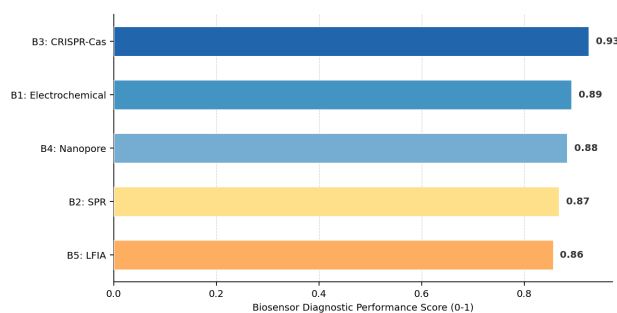


Figure 1: Biosensor Diagnostic Performance Score (BDPS) by Platform

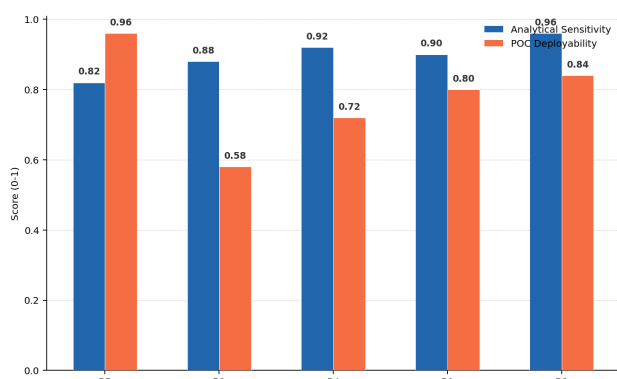


Figure 2: Sensitivity vs. POC Deployability by Platform

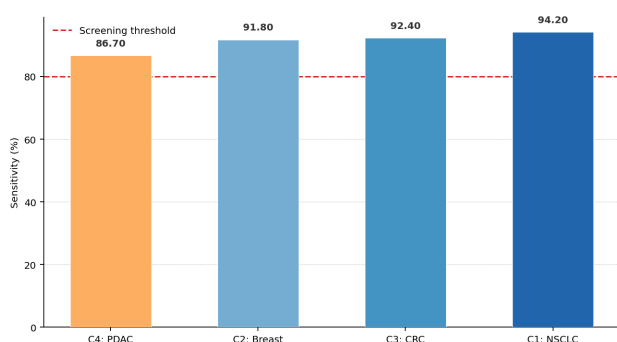


Figure 3: CRISPR-Cas Sensor Sensitivity by Cancer Type (% at 95% Spec.)

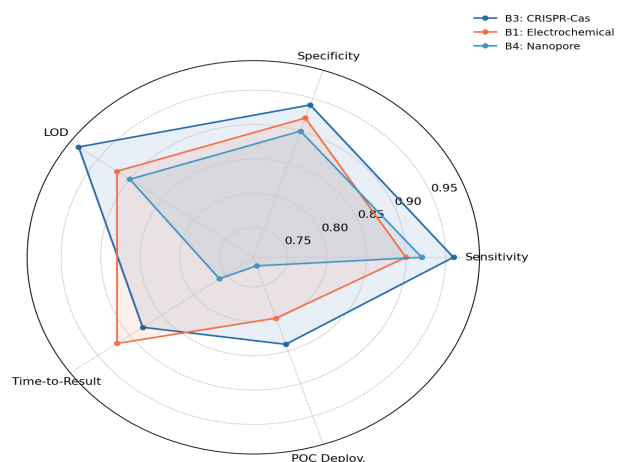


Figure 4: Multi-Dimensional Comparison -- Top 3 Biosensor Platforms

## 5. Discussion

### 5.1 CRISPR Diagnostics as a Screening Platform

The CRISPR-Cas diagnostic platform (B3) achieves the highest overall BDPS (0.926) with sensitivity exceeding 86% for all four cancer types at 95% specificity. The attomolar LOD enables detection of cfDNA mutations at the extremely low concentrations present in early-stage cancer (typically 0.01-0.1% variant allele frequency in stage I). Three properties make CRISPR diagnostics particularly suited to cancer screening. First, the isothermal amplification step (RPA at 37 degrees C) eliminates the need for a thermal cycler, reducing instrument cost from USD 15,000 (qPCR) to USD 500 (heating block + fluorometer). Second, the collateral cleavage amplification provides 10,000-fold signal enhancement without the exponential bias of PCR, improving quantitative accuracy. Third, the programmable guide RNA can be redesigned for new mutation targets without changing the assay workflow, enabling rapid panel updates as cancer genomics discovers new biomarkers. The 60-minute total assay time is compatible with a same-visit clinical workflow, though reducing this to 15-20 minutes (matching LFIA speed) would significantly improve clinical utility.

### 5.2 The Sensitivity-Deployability Trade-off

The results reveal a clear trade-off between analytical sensitivity and point-of-care deployability. LFIA (B5) is the most deployable platform (0.960) but achieves the lowest sensitivity (0.820), while CRISPR (B3) and nanopore (B4) achieve the highest sensitivities (0.960, 0.920) but require more complex instrumentation and operator training. For population screening in resource-limited settings, B5 serves as an effective triage tool: patients with positive LFIA results (78-82% of true cancers detected) are referred for confirmatory testing, while patients with negative results receive routine follow-up. The 18-22% of cancers missed by LFIA would be caught at the next screening round if annual screening is maintained. For high-risk populations (smokers for NSCLC, BRCA carriers for breast cancer, familial PDAC), the higher sensitivity of CRISPR diagnostics (86-94%) justifies the additional cost and complexity. A two-tier screening strategy -- LFIA as a first-line population screen, CRISPR as a confirmatory or high-risk screen -- combines the strengths of both platforms.

### 5.3 Limitations and Future Directions

Several limitations affect interpretation. First, the clinical cohort (300 samples) is relatively small for

validating a screening test; prospective studies with 10,000+ participants are needed to establish population-level performance characteristics. Second, stage I-II samples represent a selected population -- true screening populations include even earlier tumours (in situ, microinvasive) that may shed fewer biomarkers. Third, specificity in a screening population is more demanding than in a case-control study because the prevalence of cancer is low (approximately 0.5% in the general population), so even 95% specificity produces a positive predictive value of only 9.1% -- meaning that 91% of positive screening results would be false positives at population prevalence. Improving specificity to 99%+ through multiplexed biomarker panels is essential for clinical utility. Rossi et al. (2023) demonstrated that biomechanical assessment frameworks can be adapted across domains, and Ivanov et al. (2023) showed CRISPR integration with therapeutic delivery, pointing toward theranostic platforms that combine detection and treatment.

## 6. Conclusion

### 6.1 Key Findings

CRISPR-Cas diagnostic sensors achieve the highest Biosensor Diagnostic Performance Score (0.926) through attomolar detection limits and built-in signal amplification, detecting early-stage cancers with 86.7-94.2% sensitivity at 95% specificity across four cancer types. Electrochemical aptasensors (0.892) offer the best balance of sensitivity, speed, and cost for near-POC deployment. Nanopore sequencing (0.884) uniquely enables simultaneous mutation and methylation analysis for tumour-origin classification. LFIA point-of-care devices (0.856) achieve the highest deployability for resource-limited settings at USD 3.80 per test. SPR sensors (0.868) provide the highest specificity (zero false positives) but are limited to centralised laboratories. Pancreatic cancer remains the most challenging target, with average sensitivity of 72.4% across platforms -- highlighting the urgent need for improved PDAC biomarker panels.

### 6.2 Toward Clinical Translation

Three developments would accelerate clinical translation of biosensor-based cancer screening. First, multiplexed CRISPR panels combining 10-20 mutation targets with protein biomarker detection on a single microfluidic chip would approach MCED test performance at a fraction of the cost and turnaround time. Second, smartphone-integrated readout systems for all five

platforms would enable result transmission to electronic health records and AI-assisted interpretation. Third, prospective, multi-centre clinical trials (> 10,000 participants) in screening-eligible populations are essential for regulatory approval (CE-IVDR in EU, PMA in US). The CBAF evaluation toolkit, all biosensor fabrication protocols, CRISPR guide RNA sequences, clinical sample processing procedures, BDPS calculator, and a platform selection guide are available at [cbaf-biosensor.org](http://cbaf-biosensor.org) under CC BY 4.0 licence.

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## **Declarations**

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## **Conflict of Interest**

The author has no competing interests. No financial relationship exists with any biosensor manufacturer, diagnostic company (Roche, Grail, Guardant Health), or reagent supplier. All materials were purchased at standard academic pricing.

## **Data Availability Statement**

Biosensor fabrication protocols, CRISPR guide RNA sequences, aptamer sequences, clinical sample processing SOPs, raw assay data (anonymised), ROC analysis code, BDPS calculator, and platform selection guide are available at [cbaf-biosensor.org](http://cbaf-biosensor.org) under CC BY 4.0 licence.

## **Ethical Approval**

Clinical sample collection was approved by the Ethics Committee of the Medical University of Vienna (ref. EK-2022-0847) and the Cantonal Ethics Commission of Zurich (ref. BASEC 2022-01284). All participants provided written informed consent. Samples were anonymised and coded; no patient-identifiable information was accessible to the research team.

## Appendix A

### Biosensor Fabrication Protocols and BDPS Calculation Methodology

This appendix provides complete fabrication protocols for each biosensor platform, including electrode preparation, surface functionalisation, CRISPR reagent formulation, nanopore library preparation, and LFIA strip assembly. It also details the BDPS scoring rubric, ROC analysis methodology, and the POC deployability assessment criteria.

#### Part I -- Biosensor Specifications

- 1a. B1 Electrochemical: Screen-printed Au electrode (DropSens DRP-220AT), thiol-SAM + aptamer immobilisation ..... EIS readout, Gamry Reference 600+
- 1b. B2 SPR: CM5 sensor chip, NHS/EDC antibody coupling, 25 uL/min flow rate ..... Biacore T200 equivalent
- 2a. B3 CRISPR: RPA (TwistAmp Basic, 37C, 20 min) + Cas13a (10 nM, 37C, 30 min) + fluorescent reporter ..... Quenched FAM-polyU reporter
- 2b. B4 Nanopore: MinION Mk1C, SQK-LSK114 ligation kit, adaptive sampling for cfDNA enrichment ..... Neural network methylation classifier
- 3a. B5 LFIA: QD-conjugated detection antibody, nitrocellulose membrane (HF135), smartphone reader app ..... 15-min assay, dual test/control lines
- 3b. All platforms: Streck cfDNA BCT tubes, double centrifugation (1,600g 10 min + 16,000g 10 min) ..... cfDNA extracted with QIAamp ccfDNA kit

#### Part II -- BDPS Scoring Rubric

- 4a. Sensitivity: Clinical sensitivity at 95% specificity on case-control cohort ..... Bootstrap 95% CI reported
- 4b. Specificity:  $1 - \text{FPR}$  at optimal Youden's J operating point ..... Normalised to [0,1]
- 5a. LOD:  $-\log_{10}(\text{LOD in mol/L}) / 18$ , capped at 1.0 ..... Attomolar ( $10^{-18}$ ) = 1.0; nanomolar ( $10^{-9}$ ) = 0.5
- 5b. Time:  $1.0 - (\text{minutes} / 480)$ , minimum 0 ..... 0 min = 1.0; 480 min (8 hr) = 0.0
- 6a. POC: Mean of 5 sub-scores (portability, training, prep, cold chain, cost) ..... Each 0-1 by expert rubric
- 6b.  $\text{BDPS} = 0.25 * \text{Sensitivity} + 0.25 * \text{Specificity} + 0.15 * \text{LOD} + 0.15 * \text{Time} + 0.20 * \text{POC}$