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CRISPR-Enabled Point-of-Care Diagnostics for Early Cancer Detection

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ABSTRACT

Cancer is the leading cause of death worldwide, accounting for nearly 10 million deaths in 2020. The early diagnosis of cancer biomarkers is essential for improving prognosis and reducing the global disease burden. However, conventional diagnostic methods depend on sophisticated instrumentation, well-trained personnel, and complex workflows, which is usually time-consuming and costly. With programmable and sequence-specific nucleic acid recognition and cleavage, clustered regularly interspaced short palindromic repeats (CRISPR)-Cas systems have emerged as powerful tools for disease diagnostics. Their integration with engineering approaches, such as microfluidic devices including paper-based microfluidics, electrochemical, and smartphone-enabled platforms, has further enhanced cost-effectiveness, portability, and automation. This review presents the molecular mechanisms of CRISPR-Cas systems, recent advances in point-of-care testing (POCT) and its compatible technologies, and their applications in early cancer detection. We also discuss key challenges and future directions to enable accessible and reliable CRISPR-enabled diagnostics for improved global cancer diagnostics and provide the potential solution for effective treatment.

1 | Introduction

Accurate and timely detection of disease biomarkers is essential for improving patient outcomes and guiding treatment strategies [1]. Among human diseases, cancer remains a leading cause of mortality worldwide, with rising incidence and death rates placing an increasing strain on healthcare systems and economies [2]. The Global Cancer Statistics report indicates over 19 million new cases and nearly 10 million deaths annually, a burden projected to grow due to aging populations, lifestyle factors, and environmental exposures [3]. Early identification of malignancies is therefore critical for effective intervention and long-term health management, highlighting the urgent need for effective diagnostic approaches.

Current cancer diagnostics primarily relies on medical imaging, tissue biopsy, and molecular biomarker analysis. Comprehensive reviews of early cancer detection strategies have been reported [4, 5], providing systematic discussion of biomarker classes, clinical validation status, and implementation challenges. Imaging methods such as computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) provide tumor visualization and staging but often lack sensitivity for early-stage detection [6]. Tissue biopsy remains the gold standard for definitive diagnosis, yet it is invasive, carries procedural risks, and may be limited by tumor accessibility and sampling heterogeneity [7]. Liquid biopsy has emerged as a minimally invasive alternative, detecting circulating tumor cells (CTCs) and other tumor-derived biomarkers in bodily fluids [8]. Early cancer

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detection remains a major clinical challenge due to the low abundance of disease-associated biomarkers, tumor heterogeneity, and the limited sensitivity of diagnostic approaches. These limitations highlight the need for diagnostic approaches that combine high sensitivity and specificity with accessibility for early cancer detection.

Molecular biomarkers such as circulating tumor DNA (ctDNA) and microRNAs (miRNAs) have further attracted increasing attention as minimally invasive indicators for early cancer detection. Comprehensive reviews of cancer diagnostics have also summarized clinically actionable mutation panels and epigenetic biomarker strategies that have already undergone clinical validation [9–13]. These resources provide a practical roadmap for selecting clinically relevant genomic targets suitable for translation into molecular cancer diagnostics. Conventional molecular diagnostic techniques, such as cell culture assays, antibody-based immunoassays (e.g., enzyme-linked immunosorbent assay, ELISA), and nucleic acid detection methods, including polymerase chain reaction (PCR) and next-generation sequencing (NGS), have demonstrated high sensitivity and specificity [14]. High-throughput sequencing and digital PCR have enabled ultra-sensitive mutation and methylation detection in liquid biopsy samples, while artificial intelligence (AI)-assisted imaging analysis has improved early lesion identification. However, these methods often require sophisticated laboratory infrastructure, expensive instrumentation, and specialized personnel, limiting their applicability in resource-limited settings.

In this context, clustered regularly interspaced short palindromic repeats (CRISPR)-enabled point-of-care (POC) diagnostics offer a complementary strategy by combining programmable molecular recognition with portable detection formats. CRISPR-based approaches may complement sequencing, immunoassays, and imaging by offering point-of-care testing (POCT). POCT aims to offer rapid, accessible, and easy-to-use diagnostic solutions [15]. The World Health Organization (WHO) proposed the ASSURED criteria, emphasizing affordability, sensitivity, specificity, user-friendliness, rapidity, minimal equipment, and deliverability, later updated to the REASSURED framework to incorporate real-time data connectivity, simplified sample collection, and environmental considerations [16]. Designing POCT platforms that balance analytical performance with operational simplicity remains a challenge for resource-limited settings.

CRISPR systems with associated Cas nucleases have recently emerged as potential tools for molecular diagnostics [17]. Guided by programmable RNAs, CRISPR-Cas complexes enable sequence-specific recognition and cleavage of nucleic acids, providing high accuracy and adaptability. Cas variants such as Cas9, Cas12, and Cas13 have been investigated for detecting low-abundance targets. Integration with nucleic acid amplification strategies can further improve sensitivity, as exemplified by platforms including SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing), DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter), and HOLMES (One-Hour Low-cost Multipurpose highly Efficient System) [18–20].

Advances in microfluidics and paper-based microfluidics have further expanded the potential of POCT by enabling compact and low-cost diagnostic devices [21, 22]. Automated fluid transport is

achieved using various driving mechanisms. Coupling CRISPR-enabled detection with microfluidic control, electrochemical sensors, or smartphone-integrated readouts allows for rapid, real-time, and high-throughput analysis [23]. Although many systems were initially validated using model nucleic acid targets, the engineering principles are transferable to cancer-associated biomarkers. These systems facilitate sample processing, target amplification, and signal detection within a portable format, offering significant advantages for early cancer diagnostics. It is noted that rigorous validation, standardization, and compliance with REASSURED principles are essential before clinical translation.

This review provides a comprehensive overview of CRISPR-Cas-based POCT systems for early cancer diagnostics. We first review the molecular mechanisms of various CRISPR-Cas systems, followed by an examination of CRISPR-mediated diagnostic strategies and recent advances in POCT-compatible platforms, including microfluidic and paper-based CRISPR devices, electrochemical biosensors, and smartphone-integrated or wearable CRISPR sensors. We further discuss the application of CRISPR technology in early cancer detection and screening, highlight current technical and translational challenges, and propose future directions for implementing CRISPR-enabled diagnostics in resource-limited settings.

2 | Molecular Mechanisms of CRISPR-Cas Systems

A CRISPR-Cas system is composed of CRISPR and associated Cas nucleases, which serves as a platform for precise nucleic acid recognition and manipulation [24]. Guided by CRISPR RNAs (crRNAs), Cas effectors can selectively bind and cleave complementary DNA or RNA sequences. In addition to direct cleavage (cis-cleavage), some Cas proteins exhibit collateral activity, indiscriminately cutting nearby single-stranded nucleic acids upon activation (trans-cleavage). This feature has been widely applied in molecular diagnostics, as it enables signal amplification through the degradation of labelled reporter probes. The specificity of target recognition is dictated by crRNA, typically 17–20 nucleotides in length.

CRISPR-Cas systems are broadly divided into two classes based on their effector composition. Class 1 systems employ multi-protein complexes for interference, whereas Class 2 systems rely on a single multidomain protein, which simplifies their implementation in nucleic acid detection. Among Class 2 effectors, Cas9, Cas12, Cas13, and Cas14 have been explored for diagnostic applications, each possessing distinct structural features and cleavage behaviors [25]. Cas9, the canonical Type II effector, mediates double-stranded DNA (dsDNA) cleavage in a protospacer adjacent motif (PAM)-dependent manner through its HNH and RuvC nuclease domains. Nuclease-inactivated variants (dCas9) retain sequence-specific binding and provide a flexible platform for biosensing without cutting target nucleic acids. Engineered Cas9 variants with relaxed PAM requirements expand the range of accessible target sequences and enhance detection versatility.

Cas9 primarily supports cis-cleavage or binding-mediated detection, while Cas12, Cas13, and Cas14 present collateral cleavage

for signal analysis. Cas12 enzymes (e.g., Cas12a) recognize target DNA in a PAM-dependent fashion and exhibit robust trans-cleavage activity against single-stranded DNA (ssDNA) reporters following target binding [26]. In contrast, Cas13 effectors (e.g., Cas13a) target RNA and rely solely on crRNA guidance. Upon activation by complementary RNA, Cas13 triggers nonspecific cleavage of surrounding single-stranded RNAs, enabling highly sensitive RNA detection at single-nucleotide resolution, as demonstrated in SHERLOCK diagnostics [27]. Cas14 selectively cleaves ssDNA without requiring a PAM sequence. Its small size and strong collateral activity make it suitable for precise single-nucleotide discrimination, although its preference for single-stranded substrates may require upstream processing for double-stranded targets [28].

3 | CRISPR-Mediated Diagnostic Strategies

CRISPR-Cas systems, characterized by high specificity and programmability, have emerged as powerful tools in molecular diagnostics [29]. The enzymatic diversity of Cas effectors enables the detection of nucleic acids and non-nucleic acid targets when coupled with aptamers or nanobodies. It also enables sensitive identification of somatic mutations, gene fusions, and copy number alterations in ctDNA, as well as cancer-associated viral sequences and miRNAs for cancer detection. The modular nature of CRISPR systems allows for rapid adaptation to emerging cancer biomarkers through simple gRNA reprogramming, offering advantages over antibody-based assays that require months-long development. CRISPR-mediated diagnostic strategies are categorized into amplification-dependent, amplification-free, and one-tube approaches. Assay performance is determined by target recognition, nuclease choice, and signal transduction (fluorescence, colorimetry, lateral flow, or electrochemical readouts). The practical value of CRISPR-enabled diagnostics is evaluated by sensitivity, efficiency, robustness, user-friendliness, and feasibility (Tables 1 and 2).

3.1 | Amplification-Dependent CRISPR-Cas Diagnostics

Although CRISPR biosensors exhibit exceptional sequence-specific recognition capabilities, their direct application to low-abundance targets is often limited without prior nucleic acid amplification. Amplification strategies are essential for enhancing sensitivity, particularly for the identification of trace biomarkers at early stages of diseases [30]. The selection of appropriate amplification methods requires careful consideration of their compatibility with CRISPR-Cas systems. PCR remains the gold standard for nucleic acid amplification, enabling precise amplification of dsDNA through thermal cycling [31]. The integration of CRISPR-Cas systems with PCR and reverse transcription PCR (RT-PCR) has enabled the development of sensitive biosensors, reaching limits of detection in the attomolar range. PCR-CRISPR systems have proven effective for detecting EGFR mutations in non-small cell lung cancer (NSCLC) and human papillomavirus type 16 (HPV16) in cervical cancer [32, 33]. However, the requirement for precise thermal cycling instrumentation restricts the use of PCR in resource-limited settings.

In contrast, isothermal amplification methods provide practical alternatives. Loop-mediated isothermal amplification (LAMP) enables rapid amplification of DNA or RNA under constant temperature (typically 60°C–70°C) and a strand-displacing DNA polymerase to generate characteristic loop structures [34]. LAMP-CRISPR systems have been applied for HPV genotyping in cervical cancer screening and miRNA detection for NSCLC diagnosis [35, 36]. However, LAMP is associated with challenges such as susceptibility to nonspecific amplification. Recombinase polymerase amplification (RPA) operates at relatively low temperatures (37°C–42°C) and achieves exponential amplification through recombinase-mediated primer targeting, strand displacement, and stabilization by ssDNA-binding proteins [37]. The reaction temperatures enable the integration of RPA amplification and CRISPR detection within a single system. RPA-CRISPR systems have been employed to detect CD-1/CD-2 exosomes, inSDK1-m⁷G, and cell-free RNA (cfRNA) for colorectal cancer (CRC) diagnosis (Figure 1a) [38, 39], demonstrating body-temperature operation suitable for POC settings. Nevertheless, the lack of primer design tools and the requirement for multiple auxiliary proteins increase cost and complexity. Other isothermal amplification strategies such as rolling circle amplification (RCA), exponential amplification reaction (EXPAR), hybridization chain reaction (HCR), and recombinase-aided amplification (RAA) have also been explored [40].

In amplification-dependent CRISPR-enabled diagnostics, assay performance is largely dictated by the amplification step, as primer design, amplification efficiency, and the risk of carryover contamination influence sensitivity and accuracy. Therefore, optimization extends beyond the selection of amplification chemistry to include strategies that minimize nonspecific amplification (e.g., primer screening, hot-start systems, and contamination control). It is also important to align amplification products with CRISPR detection (e.g., amplicon length, PAM constraints, and strand accessibility). In addition, complex sample matrices often contain inhibitors that can affect amplification efficiency and Cas enzyme activity, thereby requiring reliable sample preparation strategies. Multiplexed detection also remains challenging since primer-dimer formation and cross-reactivity can interfere with CRISPR detection. Future developments should focus on universal buffer systems compatible with both amplification and CRISPR detection, as well as lyophilized reagent formats.

3.2 | Amplification-Free CRISPR-Cas Diagnostics

With the advancement of molecular detection technologies, amplification-free CRISPR-enabled diagnostic approaches have attracted increasing attention as alternatives to amplification-dependent methods. While integrating isothermal amplification with CRISPR detection into a single reaction system can reduce contamination risks and improve efficiency, it requires careful co-optimization of reaction conditions, thereby increasing technical complexity [41]. The kinetic limitations of Cas enzymes, together with background noise arising from nonspecific probe degradation, often restrict the sensitivity of CRISPR detection. Eliminating the amplification step offers advantages for POC applications, including minimal sample processing, simplified workflows, low contamination risk, and shorter time-to-result. Recent advances have focused on enhancing CRISPR signal

TABLE 1 | Representative examples of CRISPR-enabled diagnostic platforms.

Target biomarker	Cancer type	Method	Dynamic range	LOD	Time (min)	Sample	Cohort size	Sensitivity	Specificity	Reference method	Ref.
HPV16/18	Cervical cancer	LAMP-CRISPR/Cas12a	0.0031–3.1 × 10 ⁶ fM	0.0031 fM	60	Cervical cell	21	100%	100%	PCR	[35]
HPV16	Cervical cancer	Photoelectrochemical-CRISPR/Cas12a	5 × 10 ³ –5 × 10 ⁶ fM	1.2 × 10 ³ fM	120	Cervical cell	4	100%	100%	PCR	[88]
HPV18	Cervical cancer	SIMPLE-CRISPR	10 ² –10 ⁵ fM	10 ² fM	30	Cervical cell	20	90%	90%	PCR	[61]
HPV	Cervical cancer	CRISPR/Cas12a-LFA	20–2 × 10 ⁵ copies/μL	20 copies/μL	20	Clinical samples	50	90%	100%	PCR	[65]
HPV	Cervical cancer	CRISPR/Cas12a-LFA	NA	150 copies/μL	40	Plasma	150	96.7%	100%	PCR	[76]
EBV	NPC	CRISPR/Cas9 activated wearable patch	30–3 × 10 ⁴ fM	1.1 fM	75	Mouse skin	6	100%	100%	PCR	[81]
EBV	NPC	Digital-CRISPR/Cas12a	5–10 ⁵ copies/μL	5 copies/μL	60	Plasma	208	99.0%	92.6%	PCR	[89]
EGFR gene	NSCLC	PCR-CRISPR/Cas12a	0.01%–10%	0.01%	140	Plasma	140	88.1%	100%	PCR	[32]
EGFR gene	NSCLC	RPA-CRISPR/Cas12a-LFA	1–10 ⁸ copies/μL	1 copy/μL	60	Blood	57	100%	100%	PCR	[67]
NPM1 gene	AML	ERA-CRISPR/Cas12a	0.01%–10%	0.01%	30	Blood	108	100%	100%	NGS	[51]
VEGF gene	Cancer	ELISA-CRISPR/Cas13a	4–2500 fM	0.81 fM	30	Serum	NA	NA	NA	ELISA	[107]
BRCA1 gene	Breast cancer	Multicolor-CRISPR/Cas12a	5 × 10 ⁴ –10 ⁸ fM	3 × 10 ⁴ fM	60	Serum	4	100%	100%	PCR	[118]
KRAS DNA	Lung cancer	RPA-CRISPR/Cas12a-LFA	1–10 ⁵ pg/mL	0.139 pg/mL	31	Saliva	20	100%	100%	PCR	[66]
CD-1/CD-2 exosomes	CRC	RPA-CRISPR/Cas12a	10 ² –2 × 10 ⁶ EVs/μL	10 ² EVs/μL	30	Blood	100	95%	95%	PCR	[38]
PD-L1 protein	NSCLC	PER-CRISPR/Cas12a	20–10 ⁷ cells/mL	2.2 cells/mL	85	Blood	367	90%	88.0%	Flow cytometry	[58]
miR-17	Liver carcinoma	ECL-CRISPR/Cas13a	1–10 ⁵ fM	1 fM	60	Tumor cell	NA	NA	NA	PCR	[93]
miR-21	Breast cancer	MFS-CRISPR/Cas13a	10 ⁴ –10 ⁸ particles/mL	1.2 × 10 ³ particles/mL	30	Plasma	20	80%	80%	PCR	[113]
miR-122	HCC	Catalytic hairpin self-assembly-CRISPR/Cas12a	600–10 ⁶ fM	150 fM	60	Tumor cell	NA	NA	NA	Single-cell analysis	[110]
AFP	HCC	Aptamer-CRISPR/Cas12a	0.24–977 fM	0.07 fM	18	Serum	20	100%	100%	PCR	[105]
CTC	Cancer	RCA-CRISPR/Cas12a	50–10 ⁶ cells/mL	26 cells/mL	60	Blood	NA	NA	NA	Flow cytometry	[109]

TABLE 2 | Clinical validation of representative CRISPR-enabled diagnostic platforms.

Target	Cancer type	Sample	Clinical samples	Early-stage patients	Benign controls	Reference method	Ref.
HPV16/18	Cervical cancer	Cervical cell	Yes	No	Yes	PCR	[35]
HPV16	Cervical cancer	Cervical cell	Yes	No	No	PCR	[88]
HPV18	Cervical cancer	Cervical cell	Yes	No	Yes	PCR	[61]
HPV	Cervical cancer	Clinical samples	Yes	No	Yes	PCR	[65]
HPV	Cervical cancer	Plasma	Yes	Yes	Yes	PCR	[76]
EBV	EB	Mouse skin	Yes	Stage I	Yes	PCR	[81]
EBV	EB	Plasma	Yes	Stage I	No	PCR	[89]
EGFR gene	NSCLC	Plasma	Yes	No	Yes	PCR	[32]
EGFR gene	NSCLC	Blood	Yes	Stage II	Yes	PCR	[67]
NPM1 gene	AML	Blood	Yes	Yes	Yes	NGS	[51]
VEGF gene	Cancer	Serum	No	No	No	ELISA	[107]
BRCA1 gene	Breast cancer	Serum	Yes	No	No	PCR	[118]
KRAS DNA	Lung cancer	Saliva	Yes	No	Yes	PCR	[66]
CD-1/CD-2 exosomes	CRC	Blood	Yes	No	Yes	PCR	[38]
PD-L1 protein	NSCLC	Blood	Yes	Yes	Yes	Flow cytometry	[58]
miR-17	Liver carcinoma	Tumor cell	No	No	No	PCR	[93]
miR-21	Breast cancer	Plasma	Yes	No	Yes	PCR	[113]
miR-122	HCC	Tumor cell	No	No	No	Single-cell analysis	[110]
AFP	HCC	Serum	No	No	Yes	PCR	[105]
CTC	Cancer	Blood	No	No	No	Flow cytometry	[109]

output through the integration of nanomaterials (e.g., gold nanoparticles, quantum dots, and upconversion nanoparticles), which can amplify optical signals or enable alternative readout modalities, including lateral flow and electrochemical detection.

The coupling of CRISPR systems with engineered nucleic acid circuits (e.g., DNazymes and aptazymes) has expanded detection capabilities to small molecules and proteins without the need for nucleic acid pre-amplification [42]. Feedback circuits coupling signal generation with Cas enzyme activity can transform a linear cleavage process into an exponential signal amplification mechanism. As presented in Figure 1b, CONAN (CRISPR-Cas-only amplification network) employed a Cas12a-dsDNA probe system to trigger trans-cleavage activity, forming a positive feedback loop that generated exponentially increasing signals [43]. This strategy achieved attomolar-level sensitivity at 37°C using a single enzyme. Similarly, Cas13-based RNA detection systems employed self-catalytic feedback amplification mech-

anisms, employing structured RNA probes such as hairpin configurations to enhance signal output [44]. Another widely adopted strategy is the reduction of reaction volume through the use of picoliter- or femtoliter-scale droplets and microvalves [45]. This approach increases local reactant concentrations, thereby improving detection sensitivity and quantitative accuracy.

While early-stage studies have explored amplification-free strategies, future developments should undergo rigorous validation against established reference methods [42]. For instance, an amplification-free Cas12a-based aptamer fluorescence assay for prostate-specific antigen was benchmarked against ELISA. This study reported a limit of detection (LOD) of 0.16 ng/mL and a working range of 0.35–5 ng/mL, demonstrating comparable performance to ELISA (LOD 0.19 ng/mL; range 0.31–20 ng/mL), while offering operational simplicity as an advantage. Protein engineering approaches aimed at accelerating Cas/crRNA interaction kinetics are further advancing the field of amplification-

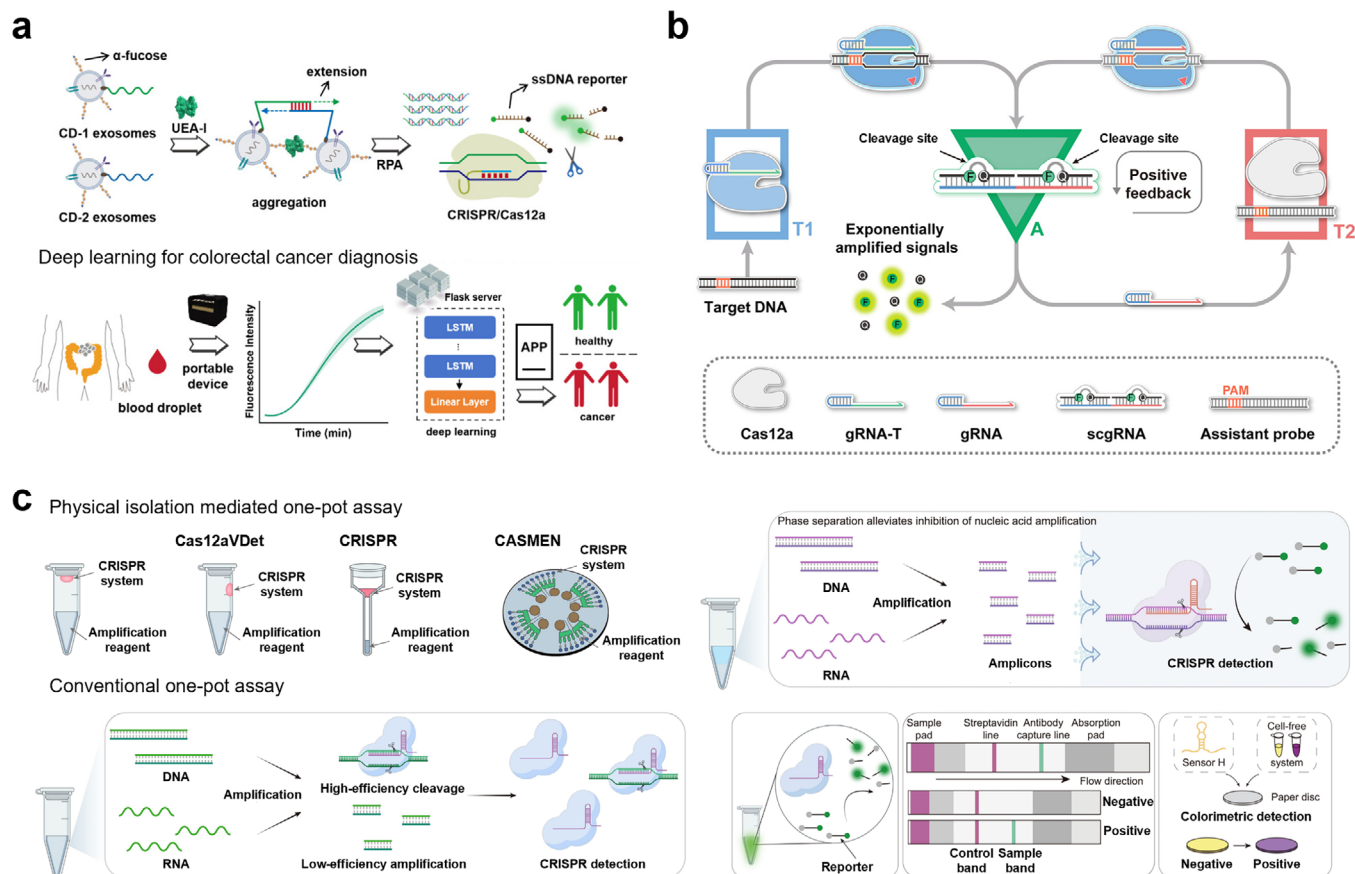


FIGURE 1 | Overview of CRISPR-mediated diagnostic strategies. (a) Deep learning-assisted POCT platform integrating lectin-induced RPA and CRISPR/Cas12a for accurate colorectal cancer diagnostics [38]. (b) CRISPR-Cas autocatalysis-driven feedback amplification network for supersensitive DNA diagnostics [43]. (c) Recent advances in one-pot CRISPR-based diagnostic strategies [49].

free CRISPR-Cas diagnostics [46]. It is noted that the implementation of amplification-free CRISPR-enabled diagnostics depends on achieving clinically relevant sensitivity and maintaining high specificity. To overcome these limitations, emerging strategies also include the development of engineered reporters, digital quantification, and computational analysis.

3.3 | One-Pot CRISPR-Cas Diagnostics

Single-tube detection technologies integrate nucleic acid amplification and CRISPR-Cas detection within a unified reaction system, thereby simplifying workflows and reducing the risk of cross-contamination. In these systems, it is necessary to simultaneously optimize the sensitivity and specificity of both amplification and CRISPR-Cas detection. Differences in reagent composition, buffer conditions, and reaction temperatures between these two processes should be carefully balanced [47]. The high temperatures required for LAMP (55°C–70°C) are often incompatible with the thermal stability of conventional Cas proteins, which has driven the development of thermostable variants such as AapCas12b [48]. Single-tube detection strategies are categorized into sequential and parallel reaction modes [49]. Sequential approaches rely on physical separation, such as layered configurations within reaction vessels, to temporally control the progression of amplification and CRISPR reactions (Figure 1c). Parallel strategies require meticulous optimization

of reaction conditions to ensure compatibility between the two processes. Recent studies have introduced approaches to balance amplification and detection, including delaying the formation of the Cas/crRNA complex [50].

The single-tube detection of RNA targets presents additional challenges, as reverse transcription and residual RNA-cDNA hybrids may inhibit amplification efficiency. Incorporation of RNase inhibitors has reduced these effects and improve sensitivity. An enzymatic recombinase amplification (ERA)-CRISPR/Cas12a assay employing engineered AsCas12a mutants was reported for acute myeloid leukemia (AML) diagnosis [51]. The method detected mutations at 0.01% sensitivity within 30 min, and identified 30 nucleophosmin 1 (NPM1) mutations in 108 patients. The presence of an NPM1 mutation in patient at the time of initial diagnosis was validated by NGS. A photoactivation strategy using photodegradable crRNA was also introduced to allow precise temporal control of Cas12a activity under 365 nm ultraviolet irradiation [52]. This approach reduced detection times for Epstein-Barr virus (EBV) to 10–20 min, demonstrating a sensitivity of 99.1% and a specificity of 100% for nasopharyngeal carcinoma (NPC) diagnosis.

The transition toward sample-to-answer systems requires the integration of nucleic acid extraction, amplification, and CRISPR detection within closed systems. One-pot CRISPR-enabled diagnostics are suitable for POC diagnostics, as they minimize

contamination risk and enable sample-to-answer workflows, particularly when combined with simplified extraction methods. However, their widespread adoption will depend on improvements in reagent stabilization (e.g., lyophilization), tolerance to inhibitory substances, and robustness under non-laboratory heating conditions. The establishment of standardized reporting parameters (e.g., thresholds and kinetic modeling) will be critical for ensuring consistency across different settings. The incorporation of AI for automated result interpretation, together with cloud-based data sharing, could transform one-pot CRISPR-enabled diagnostics from experimental tools into scalable solutions for global health monitoring.

4 | CRISPR-Enabled Platforms for POCT

The development of CRISPR-enabled diagnostics begins with the validation of signal amplification and readout strategies using simulated samples to assess sensitivity and specificity. This is followed by optimization of target isolation in clinical samples to streamline workflows and improve efficiency [53]. Validated protocols are then adapted for integration into POCT platforms. Miniaturized platforms include microfluidic and paper-based CRISPR devices, electrochemical biosensors, and smartphone-integrated or wearable CRISPR sensors. POCT systems are necessary to balance satisfactory analytical performance with short turnaround time, operational simplicity, low cost, and robustness in clinical environments. Integration of sample preparation, reaction control, and signal readout into minimally handled systems is also essential. These requirements have driven efforts to combine CRISPR detection with compact hardware, low-power fluidics, and digital interfaces, establishing portable tools capable of delivering laboratory-grade performance.

4.1 | CRISPR-Enabled Microfluidic and Paper-Based Platforms

Microfluidic technology forms the foundation of lab-on-a-chip systems, enabling precise manipulation of fluids at the sub-millimeter scale and integrating sample preparation, amplification, and detection within a single device [54]. Various actuation strategies are developed, including pneumatic, electromagnetic, and centrifugal systems [55]. The integration of CRISPR-Cas systems with microfluidics enhances high-throughput detection [56]. CRISPR-enabled microfluidic platforms have been applied to the detection of cancer-associated miRNAs and ctDNA mutations, demonstrating high sensitivity and specificity for cancer biomarkers. A digital microfluidic (DMF) platform combining barcoded DNA with CRISPR-Cas autocatalytic signal amplification was developed to detect salivary tumor markers for early oral cancer screening (Figure 2a) [57]. The system achieved LODs of 0.01 ng/mL for carcinoembryonic antigen (CEA) and 0.03 ng/mL for matrix metalloproteinase-1 (MMP-1), both below the clinical thresholds. A dual-mode microfluidic chip incorporating primer exchange reaction (PER)-CRISPR/Cas 12a and immunofluorescence visualization was also developed for PD-L1 + CTC detection in NSCLC peripheral blood [58]. It quantified surface PD-L1 across 20–10⁷ cells/mL and showed strong clinical performance for NSCLC prognosis monitoring.

Electric- and magnetic-field-driven platforms further expand functional versatility. Isotachophoresis-based systems automated nucleic acid extraction and Cas12 detection via electric field gradients, whereas magnetically assisted volumetric bar-chart (V-chip) devices employed Cas12a-triggered nanocatalytic reactions [59]. Fully integrated systems have evolved to include dual-target chips that combine amplification and CRISPR detection through simple mechanical operations, as well as centrifugal platforms enabling parallel sample processing. Additional innovations include SIMPLE-CRISPR (sample-to-result integrated platform with magnetic-bead extraction, polydisperse droplets, low-complexity operation, and engineered smartphone readout) and DMF systems that manipulate droplets via electrowetting for pathogen detection [60, 61].

Microfluidic paper-based analytical devices (μPADs) further offer accessible and cost-effective platforms for POCT [62]. Their biocompatibility, porous structure, and capillary-driven fluid transport eliminate the need for external power. Lateral flow assay devices (LFADs) represent a mature format that has been enhanced through CRISPR-Cas integration. Typical LFADs comprise a sample pad, conjugate pad, test and control lines, and an absorbent pad, enabling analyte migration and signal generation via capillary action [63]. CRISPR-mediated trans-cleavage of labeled reporter molecules produces optical signals proportional to target concentration. For cancer diagnostics, CRISPR-LFADs have enabled detection of HPV genotypes, EGFR mutations, and KRAS variants, providing instrument-free alternatives to laboratory-based genotyping [64].

Current CRISPR-enabled diagnostics are constrained by the nonspecific trans-cleavage activity of Cas effectors, which complicates the development of multiplex assays. To address this limitation, a method termed Cas12a cis-cleavage mediated lateral flow assay (cc-LFA) was developed for cancer detection (Figure 2b) [65]. This method employed a dual-key invasive hybridization strategy to recognize sticky-end DNA products, integrated with multiplex amplification and strip detection. cc-LFA accurately detected nine high-risk HPV types with > 90% sensitivity and 100% specificity, and its automated portable operation supported POC clinical applications. Alternative nucleic acid hybridization designs yielded signal intensity inversely correlated with target concentration. Signal amplification strategies can further enhance sensitivity, including catalyzed hairpin assembly (CHA)-RPA-CRISPR/Cas9 for KRAS detection in lung cancer [66], RPA-CRISPR/Cas12a for EGFR detection in NSCLC [67], surface-enhanced Raman spectroscopy-CRISPR/Cas13a for miRNA-21 detection [68], and quantum dot nanobeads modified CRISPR/Cas-LFA for breast cancer diagnostics [69].

Microfluidic and paper-based CRISPR systems offer advantages for POC diagnostics. Microfluidic devices provide precise fluid handling, automation, and multiplexing capabilities, which are suitable for complex workflows and quantitative analysis. In contrast, paper-based devices present simplicity, low cost, and equipment-free operation, which are significant for resource-limited settings. Future progress will rely on hybrid architectures that combine the automation and quantitative precision of microfluidics with the accessibility and affordability of paper substrates. Such scalable systems could support sample-to-answer workflows for the detection of low-abundance cancer

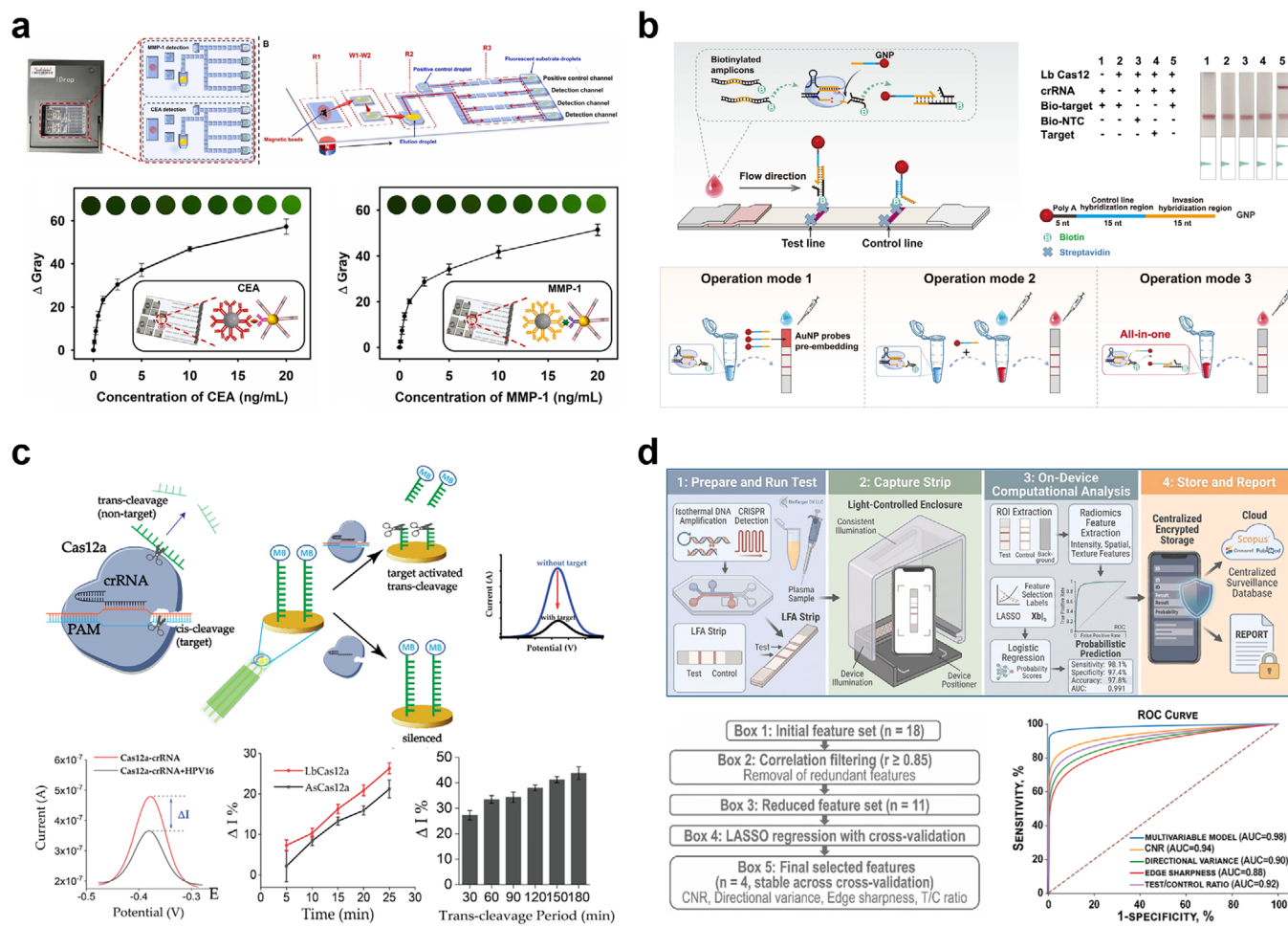


FIGURE 2 | CRISPR-enabled platforms for POCT. (a) Digital microfluidic platform integrating CRISPR-Cas autocatalytic signal amplification for dual tumor biomarker detection in saliva [57]. (b) Multiplex and ultra-specific nucleic acid detection via Cas12a cis-cleavage-mediated lateral flow assay [65]. (c) CRISPR-Cas12a trans-cleavage-based electrochemical biosensor [70]. (d) Machine learning-enhanced smartphone CRISPR-Cas12a lateral flow platform for sensitive circulating HPV DNA detection [76].

biomarkers, thereby facilitating the implementation of CRISPR-enabled POC diagnostics in early cancer screening. Despite these advances, several challenges remain in the translation of microfluidic and paper-based CRISPR-enabled platforms. Integrating multiple functional modules (e.g., sample preparation, nucleic acid amplification, CRISPR reaction, and signal readout) can introduce complexity that compromises robustness. Variability in paper matrix properties (e.g., pore size distribution and surface chemistry) may affect fluid flow and reagent distribution, thereby leading to batch-to-batch inconsistencies. Similarly, the shelf-life of pre-immobilized reagents on paper remains limited. Recent strategies include modular cartridge designs that separate lyophilized reagents and incorporation of hydrogels to stabilize enzymatic components.

4.2 | CRISPR-Enabled Electrochemical Biosensors

Although CRISPR-Cas-based colorimetric methods offer instrument-free operation, they are often constrained by limited sensitivity, specificity, and quantitative accuracy. Electrochemical sensing technologies provide rapid response, high sensitivity, and excellent compatibility with miniaturized devices due to

their millimeter-scale electrode designs. An electrochemical CRISPR-Cas12a biosensor (E-CRISPR) was fabricated to detect HPV16 and parvovirus B19 (PB19) nucleic acids at picomolar sensitivity (Figure 2c) [70]. An aptamer-based cascade version further enabled TGF- β 1 protein detection in mesenchymal stem cell (MSC) samples. These characteristics are particularly attractive for detecting low-abundance cancer biomarkers during the early stages. The fundamental principle of electrochemical biosensing lies in the conversion of biorecognition events into measurable electrical signals. Current CRISPR-enabled electrochemical strategies are categorized into labeled detection, label-free detection, and nanomaterial-enhanced detection.

In labeled systems, redox-active molecules such as methylene blue (MB) are immobilized on electrodes via ssDNA probes. Upon activation of CRISPR-Cas system, probe cleavage alters the spatial relationship between redox label and electrode surface, thereby producing quantifiable signals [71]. Representative examples include E-CRISPR sensor with an LOD of 10 fM using MB-labeled probes, and E-DNA platform employing hairpin DNA structures to enhance single-nucleotide polymorphism (SNP) discrimination. Electrochemiluminescence (ECL) biosensors further reduced background interference. For instance, Met-AuNC

system employed L-methionine-stabilized gold nanoclusters as ECL emitters in combination with Fc-labeled probes to achieve HPV16 DNA detection at sub-picomolar levels [72].

Label-free approaches simplify sensor fabrication by monitoring changes in electrode surface properties, thereby eliminating the need for probe labeling [73]. Meanwhile, nanomaterial-enhanced strategies explore the physicochemical properties of metal nanoparticles, as well as reduced graphene oxide (rGO), to improve signal amplification and detection specificity [74]. Metal nanoparticles facilitate efficient probe immobilization and signal enhancement, while rGO enables selective detection based on differential interactions with single- and double-stranded nucleic acids. CRISPR-Cas-based electrochemical biosensors have evolved from simple off-chip assays to on-chip systems. The incorporation of microfluidics can enhance operational convenience, facilitating their translation into POCT applications.

Electrochemical CRISPR biosensors are attractive due to their potential for miniaturization, rapid transduction, and quantitative output. Their compatibility with printed electrodes and portable electrochemical workstations supports scalable manufacturing and mobile application. However, challenges such as electrode fouling, signal drift, and matrix interference limit their performance. Biological fluids contain electroactive species (e.g., ascorbic acid, uric acid, and dissolved oxygen), which may generate background currents and compromise specificity. Strategies such as electrode surface modification with antifouling polymers and on-board calibration circuits have proven effective in minimizing biofouling, nonspecific adsorption, and signal drift. The transition from screen-printed electrodes to laser-induced graphene electrodes offers scalable production. Future efforts should focus on standardizing readout protocols and validating electrochemical CRISPR biosensors using cancer biomarkers to support their application in early cancer detection.

4.3 | CRISPR-Enabled Wearable Smartphone-Integrated Biosensors

Smartphones, with their high-resolution cameras and computing capabilities, have emerged as powerful tools for signal acquisition and analysis in CRISPR Biosensors. In colorimetric detection, CRISPR reverse dot blot assays combined Cas12a/crRNA complexes with ssDNA probes, generating HRP-TMB-based colorimetric signals that smartphones can quantitatively analyze [75]. Smartphone platforms equipped with emission filters and laser diode excitation have been employed for result interpretation. A CRISPR/Cas12a-LFA system interfaced with a smartphone-based machine learning (ML) model was established for quantitative analysis of plasma HPV DNA (Figure 2d) [76]. Calibration on 150 plasma samples yielded optimal diagnostic performance (sensitivity: 96.7%; specificity: 100%). Droplet magnetohydrodynamic-assisted chips also integrated magnetic nucleic acid extraction, RT-RPA amplification, and CRISPR reactions to achieve efficient nucleic acid enrichment and transport [77]. Novel signal transduction strategies further expand readout flexibility, including photothermal detection correlating Cas12a trans-cleavage [78] and iPOCT involving CRISPR-smartphone detection [79]. However, these platforms remain limited by required nucleic acid extraction, poor sensitivity, and the need for full integration.

Wearable biosensors represent another approach, enabling real-time and continuous monitoring of biomarkers. These devices integrate biorecognition elements, signal transducers, and flexible substrates, and are often augmented with microfluidic modules to enable automated sample handling [80]. Skin-attached patch sensors monitor metabolites, electrolytes, and temperature, combining current sensors, ion-selective electrodes, and microwire thermometers. Microneedle patches combining CRISPR-dCas9 with graphene biointerfaces detected cfDNA, achieving 1.2 fM sensitivity and continuous monitoring for up to 10 h [81]. CRISPR microprobes operated at body temperature and performed reliable qualitative detection in mice compared to PCR, achieving 100% sensitivity and specificity for early Stage I subjects. Smart fabrics and face mask sensors incorporated lyophilized cell-free systems, RPA, and SHERLOCK, employing multiple Cas12a-gRNA complexes to achieve femtomolar-level detection within 56–78 min, supporting cloud connectivity for real-time environmental and infectious disease monitoring [82].

Smartphone-integrated and wearable CRISPR biosensors represent the convergence of molecular diagnostics, consumer electronics, and digital health. They provide portability, real-time data sharing, and personalized health monitoring, with particular relevance for chronic disease management and home testing. However, widespread application of these technologies still faces several challenges. The transmission of health-related data via smartphone applications requires adherence to regulatory frameworks such as Health Insurance Portability and Accountability Act (HIPAA) and General Data Protection Regulation (GDPR). Accuracy can also be affected by variations in ambient lighting, camera specifications, and user handling, highlighting the need for calibration algorithms. For wearable platforms, long-term biocompatibility and the prevention of inflammatory responses at the device-skin interface are critical. The development of bioresorbable materials and bioinspired adhesive interfaces offers promising solutions to improve user safety. Future systems are likely to incorporate automated calibration, ML-assisted interpretation, and wireless connectivity, transforming CRISPR biosensing into accessible tools for longitudinal cancer surveillance and early tumor detection.

5 | CRISPR-Enabled Early Cancer Detection

Early cancer detection is essential for improving patient survival, yet nearly half of diagnoses occur at advanced stages [4]. With almost 2 million new cases and over 600,000 deaths in the United States in 2023, and global incidence projected to reach 28.4 million by 2040 [83], scalable screening strategies are urgently needed, particularly in resource-limited settings. CRISPR-enabled diagnostics represent a complementary approach by enabling sensitive, programmable, and modular detection of low-abundance biomarkers. CRISPR assays can capture ultra-low abundance targets and discriminate sequence variants in complex biological matrices, positioning them as diagnostic tools for liquid biopsy and decentralized cancer screening. While analytical sensitivity is frequently emphasized in CRISPR biosensor studies, clinical applicability for early cancer detection depends on factors including validation in clinical samples, performance in early-stage diseases, discrimination from benign conditions, and consistence with reference methods. Therefore, this section evaluates the

extent to which current CRISPR-based assays have demonstrated clinical relevance and translational potential.

5.1 | Viruses and miRNAs

HPV infection is a key risk factor for cervical and head-and-neck cancers [84]. Cervical cancer incidence and mortality remain high, with WHO recommending nucleic acid testing as screening tools. Amplification approaches typically achieve attomolar detection limits, while amplification-free methods reach low picomolar sensitivity [85]. CRISPR-associated hyperbranched RCA (CART) used Cas9/crRNA complexes to target HPV16 and HPV18 L1 regions, generating circular DNA templates for isothermal amplification [45]. CRISPR genotyping PCR (ctPCR) cleaved conserved genomic regions and ligated adapters for multiplexed amplification, with successive ctPCR versions improving performance. However, it may carry Cas9 off-target risks. In contrast, Cas12a-based DETECTR explored trans-cleavage activity to degrade synthetic probes, producing fluorescent, colorimetric, or electrochemical signals detectable on lateral flow assays or microfluidic chips [86]. LFA detection of HPV16 and HPV18 DNA in plasma achieved 93% and 30% detection rates, respectively. Approaches such as digital droplet and Raman spectroscopy have further demonstrated femtomolar to attomolar sensitivity, highlighting the feasibility of CRISPR-enabled diagnostics [33, 87].

Amplification-free CRISPR strategies offer rapid and economical detection but often require signal enhancement. Portable photoelectrochemical assays employed Cas12a to modulate G-quadruplex-mediated inhibition, producing smartphone-detectable photocurrent signals [88]. No statistically significant differences were observed between the assay and PCR at the significance level of 0.05. Electrochemical biosensors explored Cas12a-mediated cleavage of ssDNA reporters (LOD = 50 pM), while droplet digital CRISPR-Cas systems achieved 10–100 attomole LODs. Plasma samples from 108 healthy controls and 100 NPC patients were tested by a digital DNA assay [89]. The assay showed higher sensitivity (99.0%) and specificity (92.6%) than PCR (86.0% sensitivity, 88.9% specificity). CRISPR-enabled platforms have also demonstrated exceptional specificity in detecting SNPs and mutations. In Figure 3a, the EasyCatch method integrated restriction endonucleases with RPA-CRISPR/Cas12a to amplify rare mutant alleles, enabling rapid detection of EGFR e19del, L858R, and FLT3 D835 variants [90]. CRISPR-Cas12a biosensors also provided high specificity for EGFR T790M mutation [91]. By enabling sensitive detection of SNPs and mutations, CRISPR-enabled diagnostics holds broad potential for early cancer screening.

miRNAs are short non-coding RNA molecules approximately 19–25 nucleotides in length. It is demonstrated that the expression profiles of specific miRNAs closely correlate with cancer development and progression. The RCA-CRISPR-split-HRP (RCH) system converted target miRNAs into stem-loop structures that are recognized by dCas9 and amplified via RCA, coupled with HRP signal amplification [92]. HCR amplification was also employed to transform a single miRNA molecule into multiple DNA activators, further enhancing the sensitivity of Cas12a-based detection. Xing et al. developed a portable ECL chip (PECL-

CRISPR) that employed Cas13a-mediated RNA cleavage to trigger exponential signal amplification, achieving an LOD of 1 fM for miR-17 [93]. Tumor cells showed significantly higher miR-17 expression than normal hepatocytes, consistent with PCR. The PECL-CRISPR platform achieved favorable recoveries (88.7%–103.9%) in spiked cell lysates across serial miR-17 concentrations.

Despite these promising examples, CRISPR-based platforms targeting clinically actionable cancer mutations remain limited compared with the oncogenic alterations identified through sequencing. Several factors may contribute to this gap. Many cancer-associated mutations occur at extremely low variant allele frequencies in early-stage disease, posing stringent requirements for analytical sensitivity and allele discrimination [94]. Tumor heterogeneity and clonal evolution also complicate the selection of mutation targets suitable for population-level screening. In addition, while CRISPR assays demonstrate high specificity, single-nucleotide discrimination in complex cDNA backgrounds requires careful guide RNA design, optimization of PAM positioning, and suppression of amplification-induced errors. These barriers may partially explain why many studies remain proof-of-concept demonstrations rather than clinically validated assays.

To close this gap, future CRISPR-based mutation assays may prioritize well-defined hotspot regions rather than rare or highly heterogeneous mutations. Several categories of cancer-associated nucleic acid targets represent promising candidates for CRISPR-enabled POC diagnostics. These categories include recurrent hotspot mutations in oncogenes (e.g., KRAS G12, EGFR L858R, and BRAF V600E) [95–97], tumor suppressor gene alterations with high prevalence (e.g., TP53 mutations) [98], clinically actionable mutations guiding targeted therapy (e.g., PIK3CA and IDH1) [99, 100], cancer-specific methylation signatures (e.g., SEPT9 methylation) [101], and oncogenic viral integration sequences (e.g., HPV and HBV mutation sites) [102, 103]. These targets provide well-characterized sequence context, established clinical utility, and inclusion in existing diagnostic panels. Methylation-based early detection markers also offer an opportunity for CRISPR-enabled diagnostics when combined with bisulfite conversion or methylation-sensitive amplification strategies.

CRISPR-enabled diagnostics offer advantages for early cancer screening through the detection of oncogenic viruses and miRNAs. Their capacity to resolve single-nucleotide differences is particularly valuable for identifying viral genotypes, mutations, and miRNA signatures. However, clinical adoption will depend on management of analytical factors (e.g., sample type, RNA stability, and matrix interference). Translating CRISPR-enabled viral and miRNA detection into clinical practice remains challenging. Circulating miRNAs are prone to degradation by plasma RNases, requiring robust stabilization protocols during collection and processing. Absolute quantification is further complicated by sequence homology within miRNA families and the lack of reference materials. Combining CRISPR with self-sampling devices promises to enable POC screening, but user-collected samples should be validated against those collected by clinicians. Future research should examine whether multiplexed CRISPR detection can enhance predictive performance and establish miRNA expression profiles for different cancer types and populations to identify clinically significant miRNA signatures.

may add design complexity, they enable potential applications in POC diagnostics. Protein-to-nucleic acid signal conversion could position CRISPR-enabled platforms as alternative tools to ELISA. However, adapting CRISPR systems for protein detection introduces several challenges. Proteins cannot be amplified like nucleic acids, requiring signal enhancement strategies to achieve clinically relevant sensitivity. Due to conformational variability or interfering substances in complex matrices, aptamer-based recognition faces limitations in stability, specificity, and reproducibility. Additional issues (e.g., hook effect in competitive assays) may hinder calibration and inter-laboratory comparability. Future advances integrating CRISPR detection with mass spectrometry could establish CRISPR-enabled protein detection as a promising tool for precision oncology.

5.3 | CTCs and Extracellular Vesicles

CTCs play a critical role in tumor metastasis for monitoring cancer progression. Yang et al. developed a Cas12a-regulated multivalent double-stranded aptamer network (MDAN) sensor for CTC detection. This system synthesized multiple aptamer units on magnetic beads using RCA, forming an MDAN-Cas12a complex that enabled CTC quantification [109]. It achieved recoveries of 80%–84% for CCRF-CEM cells in spiked blood, comparable to the CellSearch system (80%–82%). A Cas12a-based dual-aptamer DNA network was designed to create a three-dimensional microenvironment for specific cell capture. In Figure 3c, an electrochemical biosensor for hepatocellular carcinoma (HCC) diagnosis was also reported to detect human hepatocellular carcinoma cells (HepG2) with an LOD of 20 cells/mL [110]. Spike recovery experiments in HepG2 cell lysates yielded recoveries of 2.9%–4.7%, confirming the reliability of cell lysate analysis. CRISPR activation (CRISPRa) technology was also applied to study CTC biology. A team at Harvard Medical School performed genome-wide CRISPRa screening on CTCs to identify genes that promoted tumor metastasis [111]. Among breast cancer patient-derived CTCs, a subpopulation exhibited distinct ribosome and protein translation signatures, which negatively correlated with clinical outcomes, suggesting that targeting highly malignant CTC subpopulation could help block metastatic progression.

Extracellular vesicles (EVs), particularly exosomes, are promising biomarkers for disease diagnosis and prognostic assessment. CRISPR strategies often use aptamers to bind cell surface receptors, converting cell-derived signals into nucleic acid triggers that activate CRISPR system. Cas12a-based exosome detection platforms employing CD63 aptamers have been reported, enabling sensitive monitoring of EV-associated biomarkers. Hu et al. developed a Cas12a-based method in which plasma-derived EVs were fused with liposomes, allowing sensitive detection of SARS-CoV-2 even in low-viral-load samples [112]. Similarly, a liposome-mediated microfluidic delivery system was created for Cas13a, termed MFS-CRISPR, which achieved detection of exosome samples with particle counts as low as 1.2×10^3 particles/mL [113]. MFS-CRISPR analysis of miR-21 in 20 plasma samples showed consistent results with PCR. It also achieved excellent diagnostic performance, with 80% sensitivity and 80% specificity for distinguishing breast cancer patients from healthy donors.

CTCs and EVs provide dynamic insights into tumor dissemination, treatment response, and metastatic potential. CTCs are extremely rare in advanced cancer, necessitating highly efficient enrichment strategies. EV analysis is complicated by heterogeneity among exosomes, microvesicles, and apoptotic bodies, which affect purity and cancer specificity. CRISPR-enabled diagnostics combine molecular recognition with signal amplification to enable sensitive detection of rare cellular or vesicular targets in blood. However, biological variability in CTC and EV release, influenced by circadian rhythms, activity, and therapy, highlights the need for standardized protocols, particularly regarding enrichment efficiency and target heterogeneity. Future translation will rely on integrating CRISPR detection with robust capture and isolation technologies. These studies will link CTCs and EVs to histopathological and imaging outcomes and patients' long-term survival.

5.4 | Small Molecules and Ions

Cancer cells exhibit characteristic metabolic reprogramming, leading to altered levels of specific metabolites, small molecules, and ions in body fluids that can serve as diagnostic and prognostic biomarkers [114]. The adaptation of CRISPR systems to sense metabolic alterations through allosteric transduction mechanisms extends their applicability for cancer monitoring. Zhang et al. developed CaT-SMelor, a high-throughput Cas12a-based small molecule detection platform that used bacterial aTFs [115]. Binding of target molecules to aTFs induced conformational changes that released dsDNA, activating Cas12a trans-cleavage to generate fluorescent signals (Figure 3d). CaT-SMelor was applied for uric acid detection in human blood and was adaptable to other small molecules by substituting different aTFs. Similarly, a CRISPR-enabled aTF regulatory fluorescence system was developed to couple Cas12a trans-cleavage with target small molecule binding [116]. This system was compatible with handheld visualization tools and smartphone-based readouts.

Advancing the versatility of CRISPR-enabled detection, Elledge et al. developed PICASSO (Peptide Immobilization via Cas9-Mediated Self-Assembly), which fused recombinant peptides to dCas9, creating a peptide library barcoded with sgRNAs [117]. This allowed site-specific DNA positioning for protein self-assembly, enabling antibody detection in patient sera and potential applications in enzyme substrate discovery, protein evolution, and biomolecular design. A Cas12a/Cas13a-mediated system was reported that employed HRP as an enzymatic reporter to enable amplification-free visual detection [118]. The sensor detected BRCA1 in spiked serum with recoveries of 96.4%–111% and RSDs of 0.93%–15.19%. PCR validation confirmed comparable results and demonstrated satisfactory reliability. The platform achieved up to 75-fold signal enhancement and femtomolar-level sensitivity, offering colorimetric, fluorescence, or luminescence readouts comparable to commercial kits.

Metal ions are critical in physiological and pathological processes. CRISPR-enabled metal ion sensing relies on three primary activation strategies, including aptamer-mediated, DNase-catalyzed, and other metal-responsive module-triggered approaches [119].

These mechanisms enable flexible and robust detection platforms for different metal targets. CRISPR biosensors have been adapted to detect cancer-relevant metal ions such as copper (elevated in multiple cancer types and correlating with tumor burden) and manganese (involved in antioxidant defense in cancer cells). A Cas12a-fDNA biosensor integrated structure-switching aptamers and RNA-cleaving DNazymes to detect ATP and Na⁺ at room temperature within 15 min, achieving a Na⁺ detection limit of 0.1 mM [120]. An anion detection system using riboswitch-Cas13a coupling has been developed [121], though their application to cancer-relevant ions (e.g., bicarbonate in pH regulation) requires further development.

The expansion of CRISPR-enabled diagnostics to cancer-associated metabolites and ions enables biochemical sensing of tumor metabolism and microenvironment. Such analytes can reflect metabolic rewiring, oxidative stress, ion imbalances, or microenvironmental alterations of specific cancer types and treatment responses. Detection relies on indirect transduction mechanisms, including aptamers, DNazymes, and allosteric proteins. However, challenges remain in achieving specificity for cancer-specific metabolites versus normal physiological variations. Physiological sodium exceeds the sensing window of CRISPR-enabled Na⁺ sensors, while metal ions are influenced by serum chelation and competing cations. Most demonstrations have also not been evaluated in clinical environments. Emerging synthetic biology approaches may overcome these limitations by engineering CRISPR-responsive elements capable of recognizing previously intractable small molecule targets. Future efforts to expand the range of cancer metabolites and improve accuracy will position metabolite-sensing CRISPR-enabled platforms for early cancer detection.

5.5 | Clinical Translation Considerations

Although CRISPR-Cas systems were originally developed as programmable nucleic acid recognition platforms, their capability has not yet been fully explored in cancer diagnostics. From a translational perspective, several recurrent genetic alterations represent initial targets for CRISPR-enabled early detection assays. Hotspot driver mutations such as KRAS mutations in colorectal and pancreatic cancers, EGFR mutations in lung adenocarcinoma, BRAF V600E in melanoma, and TP53 mutations across multiple tumor types provide well-defined sequences compatible with CRISPR-based nucleic acid recognition. In addition, minimally invasive liquid biopsy analytes, including ctDNA, miRNAs, and EV-derived nucleic acids, offer clinically relevant substrates for early detection. However, effective translation will require overcoming challenges associated with low biomarker abundance, mutation-specific discrimination, and inter-patient variability.

Efforts are therefore required to align CRISPR assay development with clinically validated molecular oncology. Promising directions include integration with pre-amplification strategies or digital partitioning methods to enable detection of low-frequency mutations, multiplexed CRISPR arrays capable of simultaneously profiling panels of driver mutations or methylation signatures, incorporation of error-correction mechanisms to distinguish mutations, alignment with established clinical mutation panels

to enhance diagnostic relevance. Advances in guide RNA engineering, enrichment technologies, and liquid biopsy integration will further improve specificity and sensitivity in complex clinical matrices. Given the expanding range of cancer genomic alterations identified through sequencing, CRISPR-enabled mutation detection represents a feasible direction.

Incorporating CRISPR diagnostic tools into integrated detection workflows may deliver an efficient analytical solution. For example, CRISPR assays could serve as rapid pre-screening tools to triage samples prior to NGS confirmation, or as validation methods to enhance mutation specificity following amplification assays. Integration with digital PCR may enable improved single-molecule quantification, while coupling CRISPR-mediated signal amplification with immunoassays could enhance protein biomarker sensitivity. Furthermore, CRISPR-based readouts may be embedded into liquid biopsy to support POCT, with positive cases referred for genomic profiling. Realizing this potential will require collaboration among molecular engineers and cancer biologists. Such strategies leverage the strengths of both centralized laboratory technologies and decentralized CRISPR-enabled platforms, thereby improving screening accuracy, accessibility, and cost-effectiveness.

6 | Conclusion and Perspectives

CRISPR technology has emerged as a promising platform for cancer diagnostics, particularly in resource-limited settings where operational simplicity, rapid turnaround, and device portability are essential. The analytical performance of CRISPR-enabled diagnostic systems is determined by multiple factors, including sample pretreatment, workflow complexity, signal transduction modality, and data-processing requirements [122]. Current CRISPR biosensors mainly employ Cas9, Cas12, and Cas13 nucleases, while engineered variants have further expanded their diagnostic capabilities. For instance, the thermostable nuclease AapCas12b can be coupled with LAMP to enable one-pot isothermal detection, whereas Cas13 variants support multiplexed RNA analysis [123]. High-fidelity Cas9 proteins and compact Cas effectors have helped reduce off-target activity and relax PAM constraints, thereby improving sensitivity and specificity [124]. Amplification-free strategies have also broadened the applicability of CRISPR-enabled diagnostics. The strength of CRISPR technology lies in its programmable nucleic acid recognition capability. When combined with guide RNA design, protein engineering, and digital droplet confinement, these approaches can achieve single-molecule-level detection for low-abundance biomarkers.

The integration of microfluidic technologies with CRISPR-Cas systems has improved the portability and automation capacity of diagnostic platforms, enabling detection of ctDNA, viral sequences, miRNAs, and protein biomarkers. CRISPR-Cas-based microfluidic devices have enabled cancer biomarker detection through hydrophobic barrier-mediated reaction chambers, whereas multichannel microfluidic chips support parallel nucleic acid analysis for multiplexed mutation profiling and gene fusion detection [125]. These platforms have extended beyond nucleic acid detection to include cancer-associated proteins, antibodies, and small molecules. However, several limitations remain in

sample pretreatment, device integration, and clinical implementation. Device integration faces challenges in fluidic control precision, manufacturing reproducibility, and user-friendly operation. False-positive signals caused by nonspecific amplification and aerosol contamination remain a major concern, particularly in highly sensitive amplification systems. Strategies such as one-pot reactions, enzymatic contamination control (e.g., UDG systems), and improved guide RNA design are necessary to enhance assay reliability.

Current efforts are directed toward the development of fully integrated platforms that combine sample preparation, CRISPR-enabled target recognition, and signal readout within a single device. For clinically relevant targets, additional challenges, including reagent stability, protocol standardization, and large-scale clinical validation, should still be resolved. However, most reported CRISPR-enabled cancer diagnostics have been validated using limited sample cohorts. Therefore, studies with large-scale populations are required to establish clinical sensitivity, specificity, and predictive value. Quality-control standards should also be clearly defined to accelerate clinical adoption. Combinatorial expression profiling of multiple targets may offer superior accuracy compared with single-marker strategies and represents a promising direction for early cancer screening [126]. Multiplexed biomarker detection, enabling simultaneous analysis of mutation profiles, epigenetic alterations, and expression signatures, will be essential to improve diagnostic robustness in heterogeneous tumors. Future research should therefore focus on advancing system integration and reducing manufacturing costs to improve the feasibility of CRISPR-enabled diagnostics.

To facilitate clinical translation, CRISPR diagnostic systems should be supported by standardized sample-processing and robust detection [127]. Simplified and contamination-resistant sample pretreatment methods are essential to minimize user intervention and reduce cross-contamination risks. The modularity of CRISPR-Cas systems allows rapid reprogramming to target newly identified cancer biomarkers or clinically relevant genetic variants. Reagent lyophilization can further improve stability, storage, and reproducibility, while integration with microfluidic chips and wearable devices enables automated sample handling and enhances detection efficiency. Coupling CRISPR platforms with ML-based analytical frameworks allows real-time data acquisition, interpretation, and reporting, thereby supporting remote monitoring and online diagnostics [128]. Prospective studies including early-stage cancers, benign disease controls, and longitudinal follow-up cohorts are necessary to establish sensitivity, specificity, and clinical feasibility.

CRISPR biosensors provide a versatile and practical solution for early cancer diagnostics. With the advantages of programmability, modularity, and compatibility with diverse signal readout modalities, CRISPR biosensors establish a powerful diagnostic platform for identifying early-stage malignancies and enabling timely clinical intervention. Advances in molecular engineering, enzyme engineering, microfluidic integration, and wearable device development will be essential to improve robustness, accessibility, and feasibility. With collaboration among molecular biologists, engineers, clinicians, and regulatory experts, CRISPR-enabled POC diagnostics hold strong potential for real-world cancer diagnostic applications.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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