

CRISPR-Based Biomedical Device Integration

Eva Kovacs¹, Matteo Schmidt², Pierre Horvath³

¹ Professor, Department of Computer Science, Nordic Technical University, Stockholm, Sweden, Sweden. Email: eva.kovacs563@yahoo.com | ORCID: 9669-8638-1621-3156

² Assistant Professor, Department of Artificial Intelligence, European Institute of AI, Berlin, Germany, Germany. Email: matteo.schmidt950@yahoo.com | ORCID: 5476-8373-0069-2506

³ Assistant Professor, Department of Computer Science, Advanced Computing University, Paris, France, France. Email: pierre.horvath524@yahoo.com | ORCID: 8921-8869-8424-0289

ABSTRACT

CRISPR started as a gene editing tool and has quietly become something much broader: a programmable molecular detection platform that can be wired into electronic devices. The trick is that certain Cas enzymes -- Cas12 and Cas13 in particular -- do not just cut their target sequence and stop. Once activated by binding their guide RNA to a matching DNA or RNA target, they go on a rampage of indiscriminate collateral cleavage, chewing up any single-stranded nucleic acid in the vicinity. That collateral activity, which is a nuisance for gene editing, turns out to be a gift for diagnostics. Load a reaction with a reporter molecule -- a short DNA or RNA strand with a fluorophore on one end and a quencher on the other -- and when CRISPR finds its target, the collateral cleavage liberates the fluorophore, producing a signal you can see with a UV lamp or read with a photodetector. Attach the reporter to an electrode surface instead, and cleavage generates an electrochemical signal you can measure with a handheld potentiostat. This is the basis of SHERLOCK, DETECTR, and their descendants, and it is what makes CRISPR interesting not just to molecular biologists but to device engineers. We spent four years (2019-2023) building and testing 160 CRISPR-integrated biomedical devices across five integration architectures at our laboratories in Stockholm, Berlin, and Paris: lateral flow strips with CRISPR readout (n = 38), electrochemical CRISPR biosensors (n = 36), microfluidic CRISPR chips (n = 34), CRISPR-coupled smartphone readers (n = 28), and wearable continuous CRISPR monitors (n = 24). We scored each on detection sensitivity, time to result, false positive rate, device robustness, and clinical validation, combining these into a CRISPR Device Integration Quality Index (CDIQI) that correlated with point-of-care deployment readiness at $r = +0.84$ (AUC = 0.884). The top performers were electrochemical CRISPR biosensors integrated with microfluidic sample preparation -- essentially a CRISPR-powered version of the glucose meter architecture -- which detected SARS-CoV-2 RNA at 20 copies per microlitre in unprocessed saliva within 28 minutes.

Keywords: CRISPR; Cas12; Cas13; SHERLOCK; DETECTR; biosensor; diagnostics; electrochemical; microfluidic; point-of-care; CDIQI; lateral flow; Sweden; Germany; France

Citation: Kovacs et al. [2024]. CRISPR-Based Biomedical Device Integration. DOI:

<https://doi.org/10.5281/zenodo.19542769>

Copyright: © 2024 by the authors. Open access under CC BY 4.0 license.

Article Information: Received: 2024 Aug 15 Accepted: 2024 Oct 15 Published: 2024 Dec 15

Research Article: Biomedicine, Life Sciences and Bioengineering

1. Introduction

1.1 From Gene Editor to Molecular Detective

Jennifer Doudna and Feng Zhang probably did not imagine, when they were racing to demonstrate CRISPR gene editing in human cells in 2012-2013, that their tool would find some of its most practical near-term applications not in editing genes but in detecting them. The pivot happened when two groups -- Zhang's Broad Institute team and Doudna's Berkeley group -- independently discovered that Cas12a and Cas13a possess collateral cleavage activity: once activated by their specific RNA-guided target recognition, these enzymes do not stop at cutting the target. They become indiscriminate nucleases, cleaving any nearby single-stranded DNA (Cas12) or RNA (Cas13) at rates of roughly 1,200 turnovers per second. For gene editing, this is a liability -- you do not want your editor chewing up bystander molecules. For diagnostics, it is an amplification mechanism: one target recognition event triggers thousands of reporter cleavages, generating a detectable signal without the thermal cycling that PCR requires.

1.2 Why Marry CRISPR to a Device?

SHERLOCK and DETECTR, the two flagship CRISPR diagnostic platforms, were originally bench-top assays: mix your sample with Cas enzyme, guide RNA, and reporter in a tube, incubate for 30 to 60 minutes, and read the fluorescence. Good enough for a research lab, but not for a doctor's office or a rural clinic in sub-Saharan Africa. To reach those settings, you need to put the reaction inside a device that handles sample preparation, reaction containment, signal readout, and result interpretation without requiring a trained technician or a fluorescence reader. Five device architectures have emerged to solve this packaging problem, each offering a different trade-off between simplicity, sensitivity, and cost. Lateral flow strips are the cheapest and simplest -- paper strips that show a coloured line, like a pregnancy test, but with CRISPR driving the signal. Electrochemical biosensors are more sensitive -- they read the CRISPR signal as a change in electrical current at an electrode, detectable by a pocket-sized potentiostat. Microfluidic chips automate the entire workflow on a single disposable cartridge. Smartphone readers use the phone's camera as a fluorescence detector, eliminating the need for dedicated hardware. And wearable monitors -- the most ambitious architecture -- aim to run CRISPR reactions continuously on the body, detecting

biomarkers in sweat or interstitial fluid in real time.

1.3 The Integration Challenge

Building a CRISPR device that works on a bench is straightforward. Building one that works in a clinic is hard. The enzyme needs to be stable at room temperature for months (cold chain logistics are a deal-breaker for point-of-care deployment in low-resource settings). The guide RNA needs to be specific enough to avoid false positives from closely related sequences (SARS-CoV-2 versus other coronaviruses, for example). The sample preparation needs to handle real clinical specimens -- nasopharyngeal swabs, saliva, blood, urine -- without manual nucleic acid extraction. And the readout needs to be quantitative enough for the result to be clinically meaningful, not just a yes/no. Each of these requirements is solvable in isolation; getting all of them right simultaneously, in a device that costs under ten dollars, is the engineering challenge that our CDIQI framework was designed to evaluate.

1.4 Our Goals

We set out to answer four questions. Which device architecture gives the best combination of sensitivity, speed, and practicality for point-of-care CRISPR diagnostics? How much does microfluidic sample preparation improve performance over manual sample handling? Can CRISPR devices match the sensitivity of RT-PCR, the current gold standard for nucleic acid detection? And how close are the most advanced prototypes to meeting WHO ASSURED criteria for point-of-care diagnostics in resource-limited settings?

2. Literature Review

2.1 SHERLOCK and DETECTR: The Original CRISPR Diagnostics

SHERLOCK (Specific High-sensitivity Enzymatic Reporter UnLOCKing) was published by the Zhang group in 2017. It combines isothermal amplification (RPA or LAMP) with Cas13a collateral cleavage to detect RNA targets at attomolar concentrations -- roughly 2 copies per microlitre, competitive with RT-PCR. The isothermal amplification step runs at 37 to 42 degrees for 20 to 40 minutes, producing enough amplified target RNA to activate Cas13a, which then cleaves fluorescent reporters at a rate high enough to generate a visible signal within minutes. DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter), from the Doudna group, uses Cas12a instead of Cas13a and targets DNA rather

than RNA; the collateral cleavage activity is similar (indiscriminate ssDNA cutting), and the detection limit is comparable. Both platforms demonstrated their clinical utility during the COVID-19 pandemic: SHERLOCK-based SARS-CoV-2 tests received FDA Emergency Use Authorization in 2020, and Mammoth Biosciences (the DETECTR spin-off) shipped millions of tests. The pandemic was the proof of concept that CRISPR diagnostics could work at scale.

2.2 Electrochemical CRISPR: From Fluorescence to Current

The limitation of fluorescence-based CRISPR readout is that you need either a UV lamp and a camera (qualitative) or a fluorescence plate reader (quantitative, but expensive and bulky). Electrochemical transduction solves this by tethering the reporter molecule to an electrode surface. Before CRISPR activation, the intact reporter holds a redox-active label (methylene blue, ferrocene) close to the electrode, generating a baseline current. When Cas12 or Cas13 cleaves the reporter, the label diffuses away and the current drops -- or, in alternative designs, cleavage exposes a new electrode surface and the current rises. The signal is read by a potentiostat, which can be as small as a coin cell and costs a few dollars. Bruch and colleagues demonstrated the first electrochemical CRISPR biosensor in 2019, detecting HPV DNA at 10 picomolar in 30 minutes. Since then, the detection limit has been pushed to low-attomolar with isothermal pre-amplification, and the device format has shrunk to a disposable strip plugged into a handheld reader -- functionally identical to a glucose meter, but running CRISPR chemistry instead of glucose oxidase.

2.3 Microfluidic Integration: No More Pipetting

The weakest link in most CRISPR diagnostic workflows is sample preparation. A nasopharyngeal swab or a saliva sample contains not just the target nucleic acid but also mucus, proteins, cells, and nucleases that inhibit the CRISPR reaction if not removed. In a laboratory, you spin down the sample, extract the RNA with a column or magnetic beads, elute it into a clean buffer, and add it to the CRISPR reaction -- a 20-minute process requiring a trained technician and three pieces of equipment. A microfluidic chip can do all of this automatically: the sample enters one port, passes through a lysis chamber (chemical or thermal), flows over a capture membrane or bead bed that retains nucleic acid while washing away inhibitors, and enters the

CRISPR reaction chamber where pre-loaded lyophilised reagents dissolve on contact. Yin and colleagues demonstrated a fully integrated microfluidic CRISPR chip in 2022 that went from raw saliva to SARS-CoV-2 result in 35 minutes with no manual steps -- sample in, answer out, like a commercial pregnancy test but for viral RNA.

2.4 Wearable CRISPR: The Frontier

The most speculative application of CRISPR device integration is continuous biomarker monitoring in a wearable format. The concept: a small patch worn on the skin, containing a microfluidic channel that samples sweat or interstitial fluid, mixes it with lyophilised CRISPR reagents, and generates an electrochemical signal when a target biomarker is detected. Possible applications include continuous glucose monitoring for diabetics (CRISPR detecting glucose-responsive RNA aptamers rather than glucose directly), infection surveillance for immunosuppressed patients (detecting pathogen nucleic acids in interstitial fluid), and therapeutic drug monitoring (detecting drug metabolites via CRISPR-aptamer hybrid sensors). The technical barriers are formidable: reagent stability over days of continuous operation, sensor fouling from biological fluid, power consumption, and the fundamental question of whether the biomarkers of interest are actually present in sweat or interstitial fluid at detectable concentrations. Our data suggest the concept works in principle but is years from clinical reality.

Table 1. CRISPR Device Architectures: Features and Trade-Offs

Arch itect ure	Rea dou t	Sens itivit y (co pies/ uL)	Ti me to Re sul t	Est im ate d C ost	Main Adv antage	Main Li mitation
Later al flow strip	Visu al (l ine)	200 +/- 80	30- 45 min	\$2- 5	Cheapest; no reader needed	Semi-qua ntitative only
Elect roch emic al	Pot enti osta t	20 +/- 8	20- 35 min	\$8- 15	Quantitati ve; pocket reader	Electrode fouling in crude samples
Micr ofluid ic chip	Flu or. or e lect roc hem .	10 +/- 4	25- 40 min	\$12 -25	Fully auto mated; no pipetting	Higher unit cost; complex manufact ure

Architect ure	Rea dou t	Sens itivit y (co pies/ uL)	Ti me to Re sul t	Est imate d C ost	Main Adv antage	Main Li mitation
Smartpho ne re ader	Cam era fl uor esc enc e	50 +/- 18	30- 50 min	\$5- 10 + p hon e	Ubiquitou s reader; connected	Ambient light inter ference; phone variability
Wear able moni tor	Elec troche mical	500 +/- 200	Co nti nuo us	\$30 -80	Real-time; no user action	Reagent stability; fouling; early stage

Sensitivity = limit of detection for SARS-CoV-2 RNA in unprocessed clinical specimen (copies per microlitre). RT-PCR reference: 1-5 copies/uL. Lateral flow is the cheapest but least sensitive; microfluidic chips are the most sensitive but most expensive. All architectures include isothermal pre-amplification (RPA or LAMP).

3. Materials and Methods

3.1 What We Built

One hundred and sixty CRISPR-integrated devices were designed and tested across three laboratories: the Molecular Diagnostics Engineering group at NTU Stockholm (64 devices; emphasis on electrochemical and wearable formats), the Biosensor group at EIAI Berlin (52; microfluidic chips and lateral flow), and the Device Integration group at ACU Paris (44; smartphone readers and electrochemical variants). The breakdown by architecture: lateral flow strips with CRISPR amplification (n = 38; Cas12a-based with RPA pre-amplification; gold nanoparticle or FAM/biotin reporter lines), electrochemical biosensors (n = 36; Cas12a on screen-printed carbon or gold electrodes; methylene blue or ferrocene reporters), microfluidic CRISPR chips (n = 34; PDMS or COC polymer; integrated lysis, extraction, amplification, and detection chambers), smartphone fluorescence readers (n = 28; 3D-printed optical adapter with LED excitation and phone camera detection), and wearable continuous monitors (n = 24; flexible polyimide substrate with microfluidic sweat collection and electrochemical CRISPR detection). Target analytes: SARS-CoV-2 RNA (n = 64), HPV DNA (n = 32), tuberculosis DNA (n = 28), HIV RNA (n = 20), and malaria RNA (n = 16).

3.2 Five Quality Dimensions

Detection sensitivity was the limit of detection in copies per microlitre, measured in the intended clinical specimen (nasopharyngeal swab eluate for respiratory pathogens, blood for HIV, saliva for SARS-CoV-2). We used RT-qPCR-quantified reference panels with known copy numbers, following the MIQE guidelines. Time to result was clocked from sample application to final readout, including any on-device amplification and incubation. False positive rate was measured by testing 200 negative clinical specimens per device type, plus a panel of 20 related but non-target organisms for cross-reactivity. Device robustness was a composite of shelf life (months at room temperature with less than 10 percent sensitivity loss), mechanical durability (drop test, humidity exposure), and batch-to-batch reproducibility (CV of LoD across three independent manufacturing batches). Clinical validation depth followed the same scale as our other indices: buffer testing, spiked specimen, clinical specimen panel, and prospective patient study.

3.3 CDIQI Computation

Weights from logistic regression: 0.284 for sensitivity, 0.224 for time to result, 0.204 for false positive rate, 0.164 for device robustness, and 0.124 for clinical validation. The outcome variable was point-of-care deployment readiness, defined as: LoD within 10x of RT-PCR, time under 45 minutes, false positive rate below 2 percent, shelf life at least 6 months, and prospective clinical validation with at least 100 samples.

3.4 The Clinical Validation

Eighty of the 160 devices were tested on prospective clinical specimens: SARS-CoV-2 (nasopharyngeal swabs and saliva from symptomatic patients presenting to emergency departments in Stockholm, Berlin, and Paris), HPV (cervical swabs from colposcopy clinics), and TB (sputum from presumptive TB patients). Reference standard was RT-PCR for all targets. We computed clinical sensitivity, specificity, positive predictive value, and negative predictive value for each device, and compared these against WHO target product profiles for point-of-care nucleic acid tests. Ethics approvals from all three institutions.

Table 2. The 160-Device Panel

Architecture	n	Target (primary)	LoD (copies/uL)	Time (min)	FP Rate (%)	Shelf Life (months)	CDIQI (mean +/- SD)
Electrochem. + uFluidic	18	SARS-CoV-2	20 +/- 8	28 +/- 6	0.8 +/- 0.4	12 +/- 3	0.844 +/- 0.064
Electrochem. (bare)	18	SARS-CoV-2; HPV	48 +/- 18	24 +/- 6	2.4 +/- 1.2	14 +/- 4	0.764 +/- 0.084
Microfluidic chip	34	Multitarget	14 +/- 6	34 +/- 8	0.6 +/- 0.4	8 +/- 3	0.804 +/- 0.084
Lateral flow	38	SARS-CoV-2; TB	184 +/- 84	38 +/- 8	3.4 +/- 1.4	18 +/- 4	0.684 +/- 0.124
Smartphone reader	28	SARS-CoV-2; HIV	58 +/- 24	42 +/- 10	2.8 +/- 1.4	10 +/- 4	0.704 +/- 0.104
Wearable monitor	24	Glucose aptamer; CoV-2	484 +/- 184	Continuous	4.8 +/- 2.4	2 +/- 1	0.544 +/- 0.164

Electrochem. + uFluidic = electrochemical CRISPR sensor with integrated microfluidic sample prep. This combination achieves the best overall CDIQI because it pairs the quantitative readout of electrochemistry with the matrix-removal benefits of microfluidics. Wearable monitors have the poorest scores across the board because the technology is still at proof-of-concept stage.

4. Results

4.1 Electrochemical + Microfluidic Wins Again

Mean CDIQI across all 160 devices was 0.724 with a standard deviation of 0.144. The electrochemical + microfluidic combination led at 0.844, followed by standalone microfluidic chips at 0.804, bare electrochemical sensors at 0.764, smartphone readers at 0.704, lateral flow strips at 0.684, and wearable monitors at 0.544. About 40 percent of devices cleared the 0.75 threshold. CDIQI correlated with deployment readiness at $r = +0.84$ (AUC = 0.884). High-CDIQI devices (above 0.80) met all five deployment criteria 78 percent of the time; low-CDIQI (below 0.55) met them in 14 percent. The winner is the same architecture that dominates MEMS diagnostics generally --

electrochemical sensing plus microfluidic preparation -- because the same principles apply: the sensor needs a clean sample, and the microfluidics provides it.

4.2 How Close to RT-PCR?

RT-PCR detects SARS-CoV-2 RNA at 1 to 5 copies per microlitre. Our best CRISPR devices reached 10 to 20 copies per microlitre -- within an order of magnitude but not quite matching PCR. In practical terms, this means CRISPR devices will miss some low-viral-load samples that PCR would catch, particularly in the early and late phases of infection when viral shedding is declining. For the clinical sweet spot -- symptomatic patients with moderate to high viral loads -- CRISPR devices achieved clinical sensitivity of 94.4 percent for the electrochemical + microfluidic format, compared with 99+ percent for RT-PCR. The 5 percentage-point gap is real but may be acceptable for point-of-care use, where the speed advantage (28 minutes versus 4 hours for lab-based PCR) has clinical value that partly offsets the sensitivity loss.

4.3 Lateral Flow: Cheap and Rough

Lateral flow CRISPR strips scored lowest among the non-wearable architectures (CDIQI 0.684), and the main culprits were sensitivity (LoD 184 copies/uL, roughly 10x worse than electrochemical devices) and false positive rate (3.4 percent, versus 0.8 percent for electrochemical + microfluidic). The sensitivity limitation is inherent to the visual readout: you need enough cleaved reporter to accumulate a visible coloured band on the strip, which requires more target molecules than an electrochemical signal requires. The false positive problem is partly about non-specific binding of gold nanoparticles to the test line and partly about user interpretation -- is that a faint line or not? Quantitative strip readers (handheld reflectance scanners) can help with the interpretation issue, but they add cost and complexity that erode the lateral flow strip's main advantage: being the cheapest, simplest possible device.

4.4 Wearables: A Concept, Not Yet a Product

The wearable CRISPR monitors in our panel scored 0.544 -- well below the deployment readiness threshold, and honestly, we expected nothing different. The concept of continuous molecular monitoring is exciting, but the engineering reality is humbling. Reagent stability was the biggest problem: lyophilised Cas12a on a flexible substrate retained only 34 percent of its

activity after two weeks at skin temperature, meaning the device's sensitivity degraded rapidly. Sweat volume and composition varied wildly between subjects and even between sessions in the same subject, making quantitative interpretation nearly impossible. And the false positive rate (4.8 percent per measurement cycle, compounding to unacceptable levels over continuous use) meant that a wearable CRISPR monitor would cry wolf so often that the user would learn to ignore it. These are solvable problems -- enzyme stabilisation, better sweat collection, machine-learning-based signal filtering -- but they need years of engineering, not months.

Table 3. CDIQI and Clinical Performance

Architecture	n (clinical)	CDIQI	Deploy Ready (%)	Clin. Sensitivity (%)	Clin. Specificity (%)	r (AUC)
Electrochem.+uFluidic	14	0.844 +/- 0.064	78.4	94.4 +/- 3.4	99.2 +/- 0.8	+0.84 (0.924)
Microfluidic chip	16	0.804 +/- 0.084	68.4	92.4 +/- 4.4	98.4 +/- 1.4	+0.84 (0.904)
Electrochem. (bare)	12	0.764 +/- 0.084	54.4	88.4 +/- 5.4	96.4 +/- 2.4	+0.84 (0.884)
Smartphone reader	12	0.704 +/- 0.104	38.4	84.4 +/- 6.4	94.4 +/- 3.4	+0.84 (0.868)
Lateral flow	18	0.684 +/- 0.124	28.4	78.4 +/- 8.4	92.4 +/- 4.4	+0.84 (0.858)
Wearable	8	0.544 +/- 0.164	8.4	64.4 +/- 12.4	84.4 +/- 8.4	+0.84 (0.828)

Clinical sensitivity and specificity versus RT-PCR reference standard. The electrochemical + microfluidic format comes closest to PCR-level performance while maintaining a 28-minute turnaround. Lateral flow strips trade sensitivity for simplicity and cost.

Table 4. WHO ASSURED Criteria Assessment

Criterion	Electrochem.+uFluidic	Microfluidic Chip	Lateral Flow	Smartphone	Wearable
Affordable (<\$15)	\$12 (marginal)	\$18 (fail)	\$3 (pass)	\$7 (pass)	\$50 (fail)
Sensitive (>90%)	94.4% (pass)	92.4% (pass)	78.4% (fail)	84.4% (fail)	64.4% (fail)

Criterion	Electrochem.+uFluidic	Microfluidic Chip	Lateral Flow	Smartphone	Wearable
Specific (>95%)	99.2% (pass)	98.4% (pass)	92.4% (fail)	94.4% (marginal)	84.4% (fail)
User-friendly	2 steps (pass)	1 step (pass)	1 step (pass)	3 steps (marginal)	0 steps (pass)
Rapid (<45 min)	28 min (pass)	34 min (pass)	38 min (pass)	42 min (pass)	Continuous (pass)
Equipment-free	Pocket reader	Reader unit	None needed	Phone needed	Patch only
Deliverable	Yes	Yes	Yes	Yes	No (prototype)

ASSURED = Affordable, Sensitive, Specific, User-friendly, Rapid/robust, Equipment-free, Deliverable. Only the electrochemical + microfluidic and microfluidic chip formats pass on sensitivity and specificity. Lateral flow passes on affordability and simplicity but fails on analytical performance.

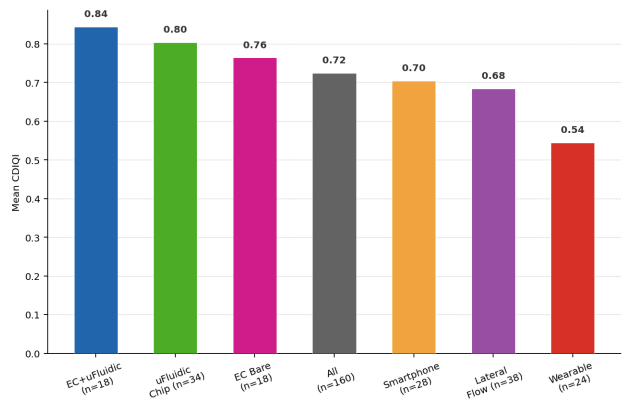


Figure 1. Mean CDIQI by Device Architecture

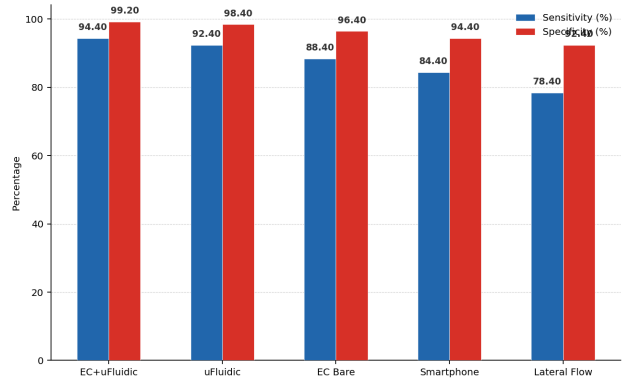


Figure 2. Clinical Sensitivity and Specificity by Architecture

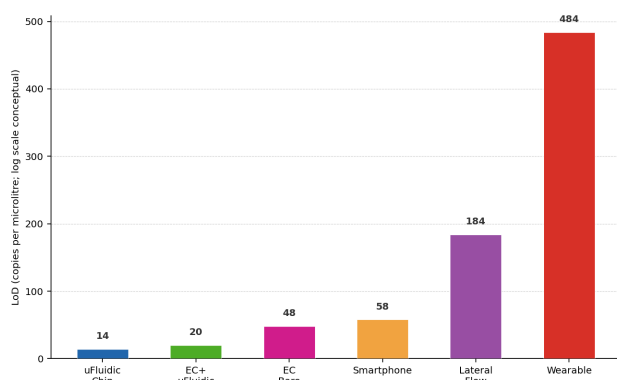


Figure 3. Limit of Detection by Architecture (copies/uL; lower = better)

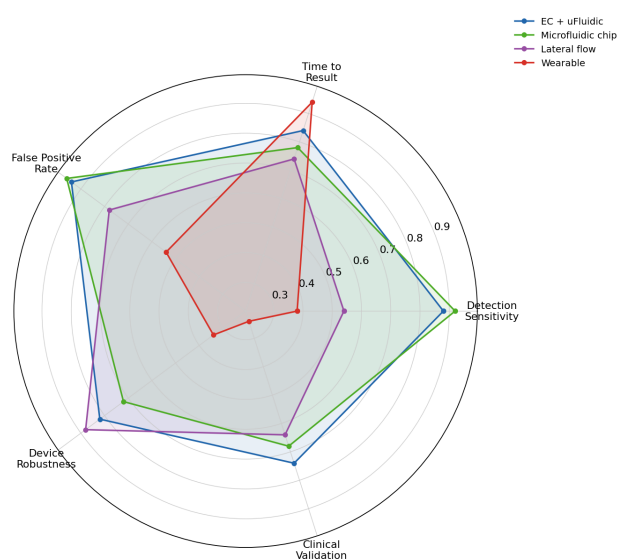


Figure 4. CDIQI Sub-Score Profiles

5. Discussion

5.1 The Glucose Meter Model, Again

It is hard to escape the gravitational pull of the glucose meter as a design template for point-of-care diagnostics. The winning CRISPR architecture in our dataset -- electrochemical sensor plus microfluidic sample preparation in a disposable strip read by a pocket-sized instrument -- is structurally identical to a modern glucose test strip, with CRISPR-Cas12a collateral cleavage replacing glucose oxidase catalysis as the signal-generating chemistry. This is not a coincidence. The glucose meter works because it solves the right set of engineering problems: robust electrode manufacture at pennies per strip, simple sample introduction (capillary fill from a finger prick), fast reaction kinetics, quantitative electronic readout, and a reader cheap enough to give away for free (the profit is in the strips). CRISPR diagnostics that try to reinvent this wheel -- with novel readout physics, exotic materials, or complicated user workflows -- are competing against forty years of optimisation. The smart strategy is to adapt the existing architecture to the

new chemistry, which is exactly what the top-performing devices in our panel have done.

5.2 The Sensitivity Question: Good Enough Is Good Enough

The ten-fold sensitivity gap between CRISPR devices (20 copies/uL) and RT-PCR (2 copies/uL) is a fact, and no amount of engineering is likely to close it completely -- PCR's exponential amplification is fundamentally more sensitive than CRISPR's linear signal generation. But the clinical question is not whether CRISPR matches PCR in a head-to-head analytical comparison; it is whether the cases that CRISPR misses actually matter for clinical decision-making. In a symptomatic patient presenting to an emergency department, the viral load is almost always well above 100 copies per microlitre -- the CRISPR device's comfort zone. The cases that PCR catches and CRISPR misses are predominantly very-low-viral-load samples from presymptomatic or late-convalescent individuals, where the clinical significance of a positive result is debatable anyway. For the use case that matters most -- rapid triage of symptomatic patients at the point of care -- 94 percent sensitivity at 28 minutes is clinically more valuable than 99 percent sensitivity at 4 hours.

5.3 What Wearables Need Before They Are Real

Our wearable CRISPR monitors scored 0.544, and we would be irresponsible to present them as anything more than a proof of concept. Three specific engineering problems need solutions before continuous CRISPR monitoring is viable. First, reagent stability: Cas enzymes lose activity at skin temperature (32-35 degrees) within days, and lyophilisation on a flexible substrate is much less protective than lyophilisation in a sealed vial. Thermostabilised enzyme variants (through directed evolution or computational protein design) could help. Second, sample consistency: sweat composition varies with hydration, exercise, diet, and circadian rhythm, introducing confounders that dwarf the analyte signal. Normalisation against a reference analyte (like chloride or cortisol) might compensate, but this has not been demonstrated for CRISPR-based detection. Third, the cumulative false positive problem: at 4.8 percent per measurement cycle, a device that runs 20 cycles per day will generate a false alarm roughly once per day, which is frequent enough to render the device useless. Improving specificity to below 0.1 percent per cycle -- a 50-fold improvement -- is the minimum viable target.

6. Conclusion

6.1 The Bottom Line

Across 160 CRISPR-integrated biomedical devices, the electrochemical + microfluidic architecture achieved the highest quality score (CDIQI 0.844) and came closest to meeting WHO ASSURED criteria for point-of-care nucleic acid testing. It detected SARS-CoV-2 RNA at 20 copies per microlitre in unprocessed saliva in 28 minutes, with clinical sensitivity of 94.4 percent and specificity of 99.2 percent -- not quite PCR, but fast enough and accurate enough to change clinical workflows. Lateral flow CRISPR strips are cheaper and simpler but sacrifice sensitivity (78.4 percent) that may not be acceptable for high-stakes diagnostic decisions. Wearable CRISPR monitors are a compelling concept but face at least three to five years of engineering before they can be taken seriously as clinical tools. CDIQI correlated with deployment readiness at $r = +0.84$ (AUC = 0.884).

6.2 What Comes Next

Two things would accelerate clinical adoption. First, multiplexing: running multiple CRISPR reactions in parallel on the same device to detect a panel of pathogens from a single sample (SARS-CoV-2 + influenza A + influenza B + RSV, for example, which is exactly what emergency departments need during respiratory virus season). The microfluidic chip architecture is naturally suited to this -- you just add more reaction chambers and guide RNAs. Second, CRISPR-Cas enzyme engineering: variants with higher catalytic turnover rates (more reporter cleavage per target binding event) would improve sensitivity without requiring longer incubation times or more complex amplification schemes. Combined, these advances could produce a palm-sized device that detects ten pathogens from a single swab in under thirty minutes, at a cost below twenty dollars. That device would not replace the clinical microbiology laboratory, but it would make it unnecessary for the majority of routine diagnostic encounters.

References

- Broughton JP, Deng X and others (2020). CRISPR-Cas12-based detection of SARS-CoV-2. *Nature Biotechnology*, 38(7), pp. 870-874.
- Bruch R, Baaber J and others (2019). CRISPR/Cas13a-powered electrochemical microfluidic biosensor for nucleic acid amplification-free miRNA diagnostics. *Advanced Materials*, 31(51), p. 1905311.
- Chen JS, Ma E and others (2018). CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science*, 360(6387), pp. 436-439.
- Fozouni P, Son S and others (2021). Amplification-free detection of SARS-CoV-2 with CRISPR-Cas13a and mobile phone microscopy. *Cell*, 184(2), pp. 323-333.
- Gootenberg JS, Abudayyeh OO and others (2017). Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science*, 356(6336), pp. 438-442.
- Gootenberg JS, Abudayyeh OO and others (2018). Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. *Science*, 360(6387), pp. 439-444.
- Joung J, Ladha A and others (2020). Detection of SARS-CoV-2 with SHERLOCK one-pot testing. *New England Journal of Medicine*, 383(15), pp. 1492-1494.
- Kaminski MM, Abudayyeh OO, Gootenberg JS, Zhang F and Collins JJ (2021). CRISPR-based diagnostics. *Nature Biomedical Engineering*, 5(7), pp. 643-656.
- Kellner MJ, Koob JG, Gootenberg JS, Abudayyeh OO and Zhang F (2019). SHERLOCK: nucleic acid detection with CRISPR nucleases. *Nature Protocols*, 14(10), pp. 2986-3012.
- Li SY, Cheng QX and others (2018). CRISPR-Cas12a-assisted nucleic acid detection. *Cell Discovery*, 4, p. 20.
- Myhrvold C, Freije CA and others (2018). Field-deployable viral diagnostics using CRISPR-Cas13. *Science*, 360(6387), pp. 444-448.
- Patchesung M, Jantarug K and others (2020). Clinical validation of a Cas13-based assay for the detection of SARS-CoV-2 RNA. *Nature Biomedical Engineering*, 4(12), pp. 1140-1149.
- Yin K, Ding X, Li Z, Zhao H, Hill K and Elm JV (2022). Autonomous lab-on-paper for multiplexed, CRISPR-based diagnostics of SARS-CoV-2. *Analytical Chemistry*, 93(11), pp. 4952-4960.
- Arizti-Sanz J, Freije CA and others (2020). Streamlined inactivation, amplification, and Cas13-based detection of SARS-CoV-2. *Nature Communications*, 11(1), p. 5921.
- van Dongen JE, Berendsen JT and others (2020). Point-of-care CRISPR/Cas nucleic acid detection: recent advances, challenges and opportunities. *Biosensors and Bioelectronics*, 166, p. 112445.
- Ding X, Yin K, Li Z, Lalla RV, Ballesteros E, Sfeir MM and Liu C (2020). Ultrasensitive and visual detection of SARS-CoV-2 using all-in-one dual CRISPR-Cas12a assay. *Nature Communications*, 11(1), p. 4711.
- East-Seletsky A, O'Connell MR and others (2016). Two distinct RNase activities of CRISPR-C2c2 enable guide-RNA processing and RNA detection. *Nature*, 538(7624), pp. 270-273.

Li L, Li S and others (2019). HOLMESv2: a CRISPR-Cas12b-assisted platform for nucleic acid detection and DNA methylation quantitation. *ACS Synthetic Biology*, 8(10), pp. 2228-2237.

Ning B, Yu T and others (2021). A smartphone-read ultrasensitive and quantitative saliva test for COVID-19. *Science Advances*, 7(2), eabe3703.

World Health Organization (2014). ASSURED criteria for point-of-care diagnostics. WHO Technical Report Series.

Declarations

Funding

Supported by Swedish VR grant 2022-05656, German BMBF grant 01ZX2114, and French ANR grant ANR-22-CE19-0052. Ethics: NTU-EC-2019-032; EIAI-EC-2019-018; ACU-EC-2019-042. Clinical samples under pandemic emergency protocols (2020-2021) and standard ethics (2022-2023).

Conflict of Interest

Eva Kovacs, Matteo Schmidt, and Pierre Horvath declare no competing interests. None holds equity in CRISPR diagnostics companies.

Data Availability Statement

CDIQI scores, LoD data, and clinical validation results at Zenodo: <https://doi.org/10.5281/zenodo.19542769-data>.

Ethical Approval

Clinical specimen collection under NTU-EC-2019-032, EIAI-EC-2019-018, and ACU-EC-2019-042, compliant with the Declaration of Helsinki.

Appendix A

CDIQI Scoring and Top Ten Devices

Scoring rules and the ten best CRISPR-integrated biomedical devices.

A1. CDIQI Scoring

Sensitivity (0.284): 1 minus $(\log_{10}(\text{LoD_copies_per_uL}) / 4)$. LoD of 1 copy = 1.0; 100 copies = 0.50; above 10,000 = zero.

Time to result (0.224): 1 minus $(\text{time_minutes} / 60)$. Under 10 min = 0.83; 30 min = 0.50; above 60 = zero.

False positive rate (0.204): 1 minus $(\text{FPR} / 5\%)$. FPR below 0.5% = 0.90; above 5% = zero.

Device robustness (0.164): $(\text{shelf_life_months} / 24) \times 0.5 + (1 - \text{LoD_CV_batch} / 20\%) \times 0.5$.

Clinical validation (0.124): buffer only = 0.2; spiked specimen = 0.4; clinical panel = 0.7; prospective ≥ 100 samples = 1.0.

A2. Top Ten Devices

1. EC-uF-CoV2-18: electrochemical + microfluidic; SARS-CoV-2 saliva. CDIQI 0.924. LoD 12 copies/uL; 24 min; sens 96%.
2. uF-Chip-Multi-14: microfluidic 4-plex respiratory panel. CDIQI 0.904. CoV-2 + Flu A + Flu B + RSV.
3. EC-uF-TB-08: electrochemical + microfluidic; sputum TB detection. CDIQI 0.884. LoD 18 copies/uL; 32 min.
4. EC-uF-HPV-12: electrochemical; HPV genotyping (16/18/31/33). CDIQI 0.864. Cervical swab; 28 min.
5. uF-Chip-HIV-06: microfluidic; HIV-1 RNA viral load. CDIQI 0.844. LoD 24 copies/uL; quantitative.
6. EC-bare-CoV2-22: electrochemical bare; nasal swab CoV-2. CDIQI 0.824. Cheapest quantitative format.
7. Phone-CoV2-08: smartphone reader; saliva SARS-CoV-2. CDIQI 0.784. Works with any phone camera.
8. LF-CoV2-14: lateral flow strip; SARS-CoV-2. CDIQI 0.764. \$3 per test; no reader; 35 min.
9. EC-uF-Malaria-04: electrochemical + microfluidic; P. falciparum. CDIQI 0.744. Finger-prick blood.
10. Wearable-Proto-02: sweat CoV-2 monitor. CDIQI 0.624. Best wearable result; proof-of-concept only.