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Current Opinion in Electrochemistry

Review Article

Towards CRISPR powered electrochemical sensing for smart diagnostics

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Abstract

Even though global health has been steadily improved, the global disease burden associated with communicable and non-communicable diseases extensively increased healthcare expenditure. The present COVID-19 pandemic scenario has again ascertained the importance of clinical diagnostics as a basis to make life-saving decisions. In this context, there is a need for developing next-generation integrated smart real-time responsive biosensors with high selectivity and sensitivity. The emergence of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas biosensing systems has shown remarkable potential for developing next-generation biosensors. CRISPR/Cas integrated electrochemical biosensors (E-CRISPR) stands out with excellent properties. In this opinionated review, we illustrate the rapidly evolving applications for E-CRISPR-integrated detection systems towards biosensing and the future scope associated with E-CRISPR based diagnostics.

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Current Opinion in Electrochemistry 2021, **30:**100829

This review comes from a themed issue on **Sensors and Biosensors (2021)**

Edited by **Sabine Szunerits**

For complete overview about the section, refer Sensors and Biosensors (2021)

Available online 12 August 2021

https://doi.org/10.1016/j.coelec.2021.100829

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Keywords

CRISPR/Cas, Electrochemical, Biosensing, Point-of-care, E-CRISPR.

Introduction

Bacteria and Archea has an adaptive immune defence system, and the discovery of the same is the greatest achievement in the field of science since the past decade. Nucleic acid-based immune mechanism helps in protecting microorganisms from viral infection and is referred to as CRISPR/Cas system (clustered regularly interspaced short palindromic repeats/CRISPR-associated systems) [1]. Since its discovery, CRISPR has been used extensively as a genome engineering tool and also for DNA or RNA recognition [2*] with specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) [3]. SHERLOCK is the first CRISPR/Cas-based sensing approach for pathogen detection and uses CRISPR/Cas13a system and recombinase polymerase amplification strategy for collateral cleavage and target isothermal amplification [4]. A paper-based CRISPR test for COVID-19 known as FnCas9 editor linked uniform detection assay (FELUDA) was recently developed by the Tata group and CSIR, India. FELUDA uses Cas9 enzymatic cleavage property to detect nucleotide sequence [5,6].

Different nucleic acid detection and amplification methods are practised worldwide [7]. To mention few, PCR, including traditional, quantitative and digital PCR [8], different isothermal techniques, such as loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification, rolling circle amplification, sequence-based amplification, nicking enzyme amplification reaction, exponential amplification reaction, and strand displacement amplification [9]. Sequencing methods such as Sanger, next-gen, nanopore sequencing and blotting techniques such as Southern and Northern have been practised. Fluorescence in situ hybridization is one of the non-amplifications cytogenic techniques used for the detection of gene mutations and the identification of microbial species. However, the fluorescence in situ hybridization system showed a poor limit of detection and background issues associated with fluorescent microscopes [10,11]. All the above-mentioned techniques gained fame in the initial phase because of better detection ability and advantages, nevertheless increasing demand for on-site point-of-care (POC) devices and associated flaws, such as low sensitivity, specificity, selectivity and relative expenses challenged traditional methods [12]. On the contrary, the advent of CRISPR paved new cost-effective opportunities and provides attomole range limit of detection, high specificity and sensitivity [2,4]. Emerging needs in monitoring disease

Table 1

Represents recent advances in E-CRISPR based biosensors for different clinical biomarkers.

CRISPR/ Cas system	Pathogen	Detection Technique	Detection limit	Sample	Year	Reference
Cas 12a	HPV16 PB-19 TGF-β1	SWV	50 pM 0.2 nm	Amplified ssDNA Spiked sample	2019	[22]
Cas 13a	miRNA-19B miRNA-20A	Amperometry	10 nM	Serum sample	2019	[33*]
Cas 9/Cas 12a	PB-19	SWV	10 fM	Serum sample	2020	[28]
Cas 12a	HPV 16, HPV 18	DPV	30 pM	Amplified ssDNA Spiked sample	2020	[30]
dCas 9	Tumour DNAs (ctDNA)	Impedence spectroscopy	0.65 nM	Blood sample	2020	[34]
Cas13a	miRNA-17	Electrochemiluminescence	1 pM	Human tumour cells	2020	[35]
Cas 12a	HPV 16, HPV 18, HIV	SWV	1.2×10^4	Cervical swab sample	2021	[27]
Cas 12a	*Listeria monocytogenes*	SWV	0.68 aM	*Listeria* in spiked sample	2021	[36]
Cas 9	Tumour DNA detection	DPV	0.13 pM	*Serum sample*	2021	[37]
dCas 9	DNA	Impedence spectroscopy	33.96 fM	*Glioblastoma*	2021	[38]
Cas 13a	Non-small-cell lung carcinoma RNAs	SWV	50 aM	*Serum sample*	2021	[39]
Cas 13a	miRNAs	DPV	2.6 fM	Serum sample	2021	[40]
Cas 12a	microRNAs PB-19 Adenosine-5′-triphosphate	SWV	0.83 aM 0.52 aM 0.46 pM	Serum sample	2021	[41]
Cas 12a	HPV-16	Electrochemiluminescence	0.48 pM	Blood sample	2021	[42]

outbreaks and patient management have prompted the development of rapid and sensitive POC devices. The term REASSURED was coined in 2018 by including two more criteria of real-time connectivity (R), and ease of specimen collection and environmentally friendly (E) to the previous WHO's ASSURED criteria, hence aiding in scaling up diagnosis even in low- and middle-income countries [12*]. By digitalizing the POC platform, data can be linked to proficiency testing by aiding in accessing patient's health information thereby reducing interpretation and transcription errors. On one hand, CRISPR-Cas systems offer perfect gene-editing tools, extreme sensitivity and programming ability makes them an apt tool for biosensing applications with minimal technical expertise and instrument clusters. On the other hand, CRISPR/Cas-based systems allow highly specific, rapid, cost-effective, multiplex alternatives to detect target nucleic acids, viruses, bacteria, cancer mutations, and proteins [13–15], which could satisfy the REASSURED criteria for diagnostics.

In general, CRISPR/Cas systems are divided into two different classes. Class 1 systems have multiple effector complexes for the target, whereas, class 2 requires a single-effector for the target. CRISPR systems are easily programmed with CRISPR-RNA's, recognized and cleaved either by single-stranded or double-stranded DNA. CRISPR-Cas system consists of guide RNA (gRNA) and CRISPR-associated (Cas) nuclease. Cas9 nuclease has guide RNA made of CRISPR RNA capable of binding to target DNA and a trans-activating RNA binding to the nuclease to regulate cleavage activity, whereas, Cas12a and Cas13a systems have only CRISPR RNA, responsible for both binding and cleavage activity. CRISPR type II, III, V, VI RNA guided nucleases (Cas 9, Cas12a, Cas13, Csm6) has been extensively applied for nucleic acid detection [16–18], (see Table 1).

Simplicity, accuracy, sensitivity, selectivity, low-cost, minimal equipment, and shorter turn-around time are highly desirable and synonyms for new age biosensors. To facilitate CRISPR/Cas multiplex biosensing, integrating CRISPR/Cas with some alternative techniques need to be considered. Compared with the existing detection strategies, electrochemical techniques are cost-effective, easy to fabricate, easy to functionalize and miniaturize, offers relatively high sensitivity and selectivity along with rapid responses, therefore extensively applied for biosensing applications [19–22]. Taken together with the advantages of the CRISPR/Cas system and electrochemical techniques, it is plausible to achieve significant improvement in biosensing with biological samples, which are usually marred by the presence of interferents, results in impeding electron transfer process and subsequent measurements. In this current opinion, we have put up our effort to opine on recent advances in the electrochemical-CRISPR–based biosensors, hurdles and future perspectives in disease monitoring and patient management.

CRISPR/Cas integrated electrochemical sensors

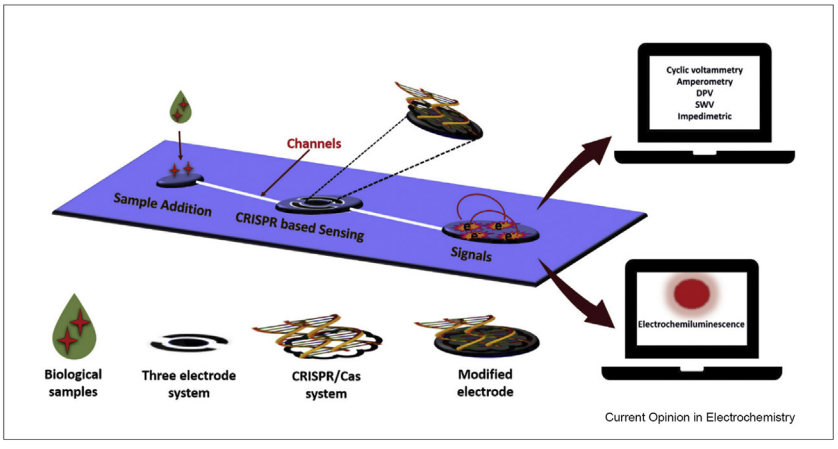
Recently, Cas integrated electrochemical sensors (E-CRISPR) has been extensively used to address the

solution for the detection of biomarkers because of high selective affinity-based interactions with varied biological targets. E-CRISPR is an electrochemistry-integrated system hence comprises of working electrode, counter electrode and reference electrode [22]. E-CRISPR-integrated POC's are the need of the hour because of versatile advantages and easiness to translate into smart devices, making clinicians analyse data in a short time and offer better care for patients. Figure 1 represents the schematics for a simple E-CRISPR-integrated POC device. In the E-CRISPR system, Cas starts cleaving reporter strands only when complementarity is formed, resulting in the formation of electrochemical outputs. This versatile and simple approach of CRISPR has been successfully integrated into developing sensors for wide biologically important analytes, such as nucleic acid, protein, pathogen detection, genome engineering, and transcription regulation [22–31].

For instance, CRISPR-Cas systems have been used for electrochemical detection of viral nucleic acids of human papillomavirus 16 (HPV- 16) [22], HIV [27] and parvovirus B19 (PB-19) [22,28,41]. The non-specific short ssDNA tagged with methylene blue (MB-ssDNA) electrochemical tag for signal transduction are commonly used [22]. Similarly, MB tag was used with hairpin DNA (hpDNA), to study the cleavage action of Cas12a for fabricating electrochemical DNA sensors [30]. In presence of the target, Cas12a trans-cleavage activity is activated, cleaving MB-ssDNA reporter off the electrode surface, therefore decreasing MB signal transducer in the form of low or off electrochemical signal. On the contrary, a high or on electrochemical signal is achieved in the absence because of silencing of trans-cleavage activity. In addition, Figure 2, demonstrate the aptamer-based E-CRIPR cascade for protein detection. In general, square wave or differential pulse voltammetry has been applied for the measurement of the electrochemical signal, with picomolar (pM) to femto molar (fM) sensitivity towards HPV [22,30] and PB-19 [22,28,41]. Whereas the combination of LAMP with E-CRISPR readout provides high sensitivity, low cost and better signal transduction render the POC device favourable for resource-poor settings. Zamani et al. demonstrated successful monitoring of 1.2×10^4 copies of HPV-18 DNA with the combination of LAMP and E-CRISPR. Moreover, the potential applicability of developed platform for clinical diagnostics was shown by the detection of HPV-18 DNA in cervical swabs with 100% sensitivity and 89% specificity [27**]. Schematic representation of integrated LAMP product with E-CRISPR technique is depicted in Figure 3. Combination of CRISPR/Cas with electrochemical DNA sensor has been used for fabricating powerful biosensing actuator system for PB-19, known to cause erythema infection in pregnant women and children. Implementation of the target recognition induced cleavage activity of CRISPR system for DNA sensor, releases electrochemical tag from sensor surface after cleavage of the target nucleic acid by Cas enzyme and results in an apparent change in an electrochemical signal. Although the developed CRISPR-DNA sensor strategy is generalizable, amplification-free, sensitive and retains accuracy based on a signal-off assay, might limit the dynamic detection range [28]. Hence focus should be given to signal-on strategy providing potential for a wide linear range and high sensitivity.

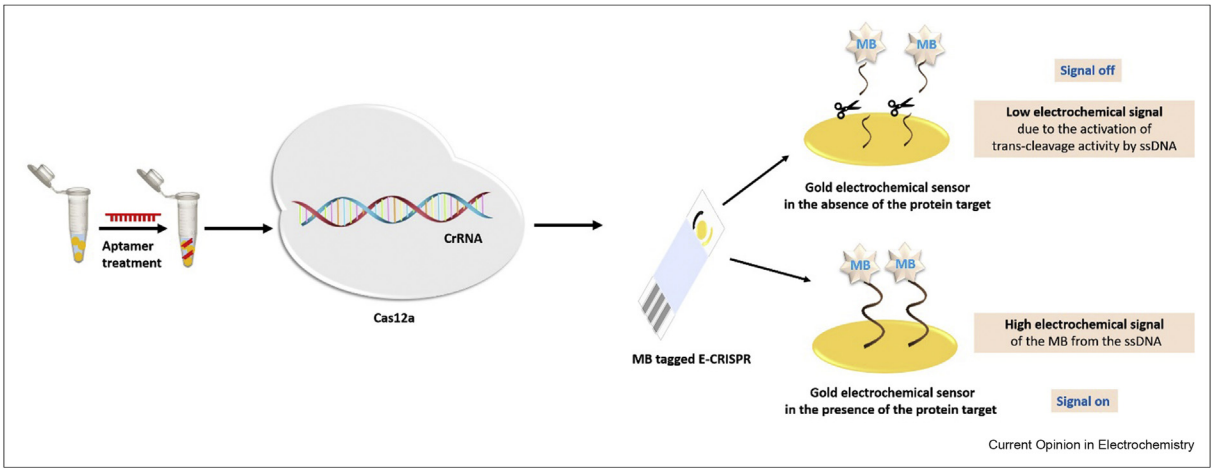
E-CRISPR integrated with microfluidics has been used for monitoring potential brain tumour maker miRNA-19b in patient serum samples. The developed

Figure 1



Schematic representation of working mechanism for E-CRISPR based POCs platform. Briefly, processed sample is added onto the sample zone, moves through the channels to reaction zone (modified with CRISPR-Cas system). The CRISPR-Cas system gets activated in presence of target and cleaves the target developing an off-signal. On the other hand, in the absence of target, CRISPR-Cas system is inactivated resulting in signal-on. The resulting signals are monitored with electrochemical or electrochemiluminescence readouts. CRISPR, clustered regularly interspaced short palindromic repeats; POC, point-of-care.

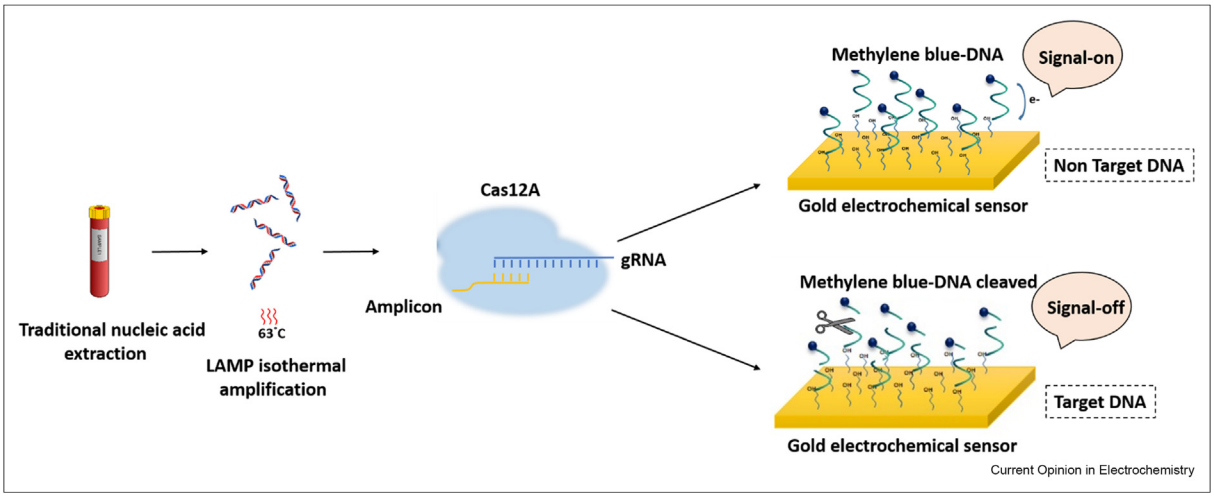
Figure 2



Aptamer-based E-CRISPR protein biosensor involving, Cas12a-crRNA specific for aptamer. In the presence of target, less aptamer is captured and transduced by E-CRISPR resulting high electrochemical signal from MB from the ssDNA reporter. Conversely, in the absence, the activation of tans−cleavage activity by ssDNA target recognition results low electrochemical signal.

microfluidic chip contains an electrochemical cell, channels, immobilization area, and is immobilized with streptavidin, functioned to capture biotin labelled RNA (6-fluorescein amidate (6FAM)-biotin-ssRNA). Here Cas13a/crRNa complex was incubated with a sample containing miRNA target and 6FAM-biotin-ssRNA label. The reaction mixture included glucose oxidase (GOx) labelled anti-fluorescein antibodies, biotin-6Fam-ssRNA, streptavidin, along uncut reporters bound to antibodies. The assay readout is performed by introducing glucose and subsequent electrochemical detection of produced H_2O_2 by the labelled GOx. It should be noted that the authors were successful in demonstrating the detection of miRNAs in a small sample volume without relying on any nucleic acid amplification steps. Moreover, the developed biosensor exhibited the ability to detect miRNA-19b up to 10 pM in buffered solutions and could measure circulating miRNAs in the clinically acceptable range. The inferences obtained from this work found that the signal-off system is sensitive enough for clinical sample analysis [26**].

Figure 3



Integrated LAMP and E-CRISPR platform on gold electrodes for biosensing applications involving traditional nucleic acid extraction followed by amplification using LAMP and subsequent activation of Cas12a that cleaves methylene blue tagged oligonucleotide immobilized on gold electrochemical sensors resulting signal-off for a target DNA and on the contrary signal-on for Cas12a inactivated non-target DNA resulting without cleavage.

Analytical performance of E-CRISPR could compromise with the placement of conventional linear ssDNA reporter on the electrode as a sensing interface, due to large electron tunnelling distance from redox labels hindering electron transfer and also steric hindrance effect on the interface causing low cleavage efficiency of Cas12a towards ssDNA. By using hpDNA in place of linear DNA, previously mentioned drawbacks can be circumvented. Zhang et al. [30] developed a hpDNA linked with methylene blue (MB) tag-based E-CRISPR biosensor for HPV DNA, where a detection limit of 30 pM in 60 min was observed.

Low-resource settings exceptionally carry the burden of an infectious disease due to lack of facilities, trained personnel, and expensive diagnostic tests; hence, Newsham and Richards-Kortum [31] stressed the need for accomplishing a greater number of clinical sample validation before translating CRISPR based electrochemical detection strategies for use in low-resource settings. Moreover, nucleic acid extraction by using paper-based sample processing and heating techniques, such as wireless resistive heaters to perform LAMP could be explored [31]. Also, long term storage can be achieved by lyophilizing CRISPR-Cas systems aiding in stability at ambient conditions [32**].

Conclusion and future perspective

Irrespective of resource-poor or resource-rich settings, real-time rapid diagnosis plays a major role in identifying future threats raised by many diseases [43]. CRISPR/Cas systems are boon and are the ultimate weapons for developing rapid molecular diagnostics to address the spread of disease, timely intervention and decision making. E-CRISPR based methods can be effectively used for developing sensors because they are most reliable, as well as highly sensitive transduction systems with less susceptibility towards impurities and provides the most selective, accurate, and reproducible performances along with easy disposable opportunities [44]. Interestingly, CRISPR technology has provided rapid, on-field, sensitive, and specific assay for SARS-CoV-2 detection with simultaneous dual-gene detection of SARS-CoV-2 in a single lateral flow strip with gold nanoprobes [45*]. The past two years have brought new insights in the field of electrochemical biosensors using CRISPR based approaches. Hence, development of E-CRISPR based biosensor along with nano enabled-probes and tags could endow the realization of rapid detection of different strains of virus at ultralow concentration. nTo reduce off-target issues, Cas14 can be incorporated into CRISPR sensing because it selectively detects single nucleotide mismatches [32]. Disease outbreaks commonly affect rural areas with low access to the health care systems for proper diagnosis and treatment; hence, E-healthcare or digital health has gained attention to endow health solutions. In addition, it is also an alternative approach for solving accessibility to healthcare system and for generating smart data for disease management. Inclusion of the internet of things, artificial intelligence and Blockchain analysis into POC sensing platform improves data curation for emerging disease scenarios, dynamics. The incorporation of computation and statistical learning tools while designing the E-CRISPR POC device will alleviate the noise and variation in signals unlike the present simple analogue to digital transition and also support decision making for development of futuristic E-CRISPR based biosensors [46**-49]. However, despite the convenience, sensitivity, and cost-effectiveness about electrochemical biosensors, most lack clinical validations. Hence, more focus should be on validating E-CRISPR sensing platforms to foster rapid disease and patient management.

Author contributions

PDPS, SJ, KS: Conceptualization, Writing-original draft, Writing-review and editing**. KSP:** Conceptualization, Supervision, Writing-original draft, Writing-review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors would like to Yenepoya (Deemed to be University), SJ and KS indebted to ICMR for Senior research Fellowship. PDPS is grateful to DST-INSPRE doctoral fellowship. KSP is thankful to DBT and DST-SERB, Government of India.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Johnston RK, Seamon KJ, Saada EA, Podlevsky JD, Branda SS, Timlin JA, Harper JC: **Use of anti-CRISPR protein AcrIIA4 as a capture ligand for CRISPR/Cas9 detection**. *Biosens Bioelectron* 2019, **141**:111361–111367.

2. * Dai Y, Wu Y, Liu G, Gooding JJ: **CRISPR mediated biosensing toward understanding cellular biology and point-of-care diagnosis**. *Angew Chem* 2020, **132**:20938–20950.
Comprehensive mini review on the need and *in vitro* / *in vivo* application for detection and bioimaging utility of CRISPR based systems.

3. Kellner MJ, Koob JG, Gootenberg JS, Abudayyeh OO, Zhang F: **SHERLOCK: nucleic acid detection with CRISPR nucleases**. *Nat Protoc* 2019, **14**:2986–3012.

4. Gootenberg JS, Abudayyeh OO, Lee JW, Essletzbichler P, Dy AJ, Joung J, Verdine V, Donghia N, Daringer NM, Freije CA, Myhrvold C: **Nucleic acid detection with CRISPR-Cas13a/C2c2**. *Science* 2017, **356**:438–442.

5. Azhar M, Phutela R, Kumar M, Ansari AH, Rauthan R, Gulati S, Sharma N, Sinha D, Sharma S, Singh S, Acharya S: **Rapid, accurate, nucleobase detection using FnCas9**. *medRxiv* 2020, https://doi.org/10.1101/2020.09.13.20193581.

6. Mukherjee K: *HTA of FELUDA diagnostic test for COVID-19*. 2020. https://www.cambridge.org/engage/apigateway/coe/assets/

orp/resource/item/5f893cf25f98de001944bff7/original/hta-of-feluda-diagnostic-test-for-covid-19.pdf.

7. Xu X, Luo T, Gao J, Lin N, Li W, Xia X, Wang J: **CRISPR-Assisted DNA detection: a novel dCas9-based DNA detection technique**. *CRISPR J* 2020, **3**:487–502.

8. Cao L, Cui XY, Hu J, Li Z, Choi JR, Yang Q, Lin M, Hui LY, Xu F: **Advances in digital polymerase chain reaction (dPCR) and its emerging biomedical applications**. *Biosens Bioelectron* 2017, **90**:459–474.

9. Zhao Y, Chen F, Li Q, Wang L, Fan C: **Isothermal amplification of nucleic acids**. *Chem Rev* 2015, **115**:12491–12545.

10. Zhang L, Ding B, Chen Q, Feng Q, Lin L, Sun J: **Point-of-care-testing of nucleic acids by microfluidics**. *Trends Anal Chem* 2017, **94**:106–116.

11. Bonini A, Poma N, Vivaldi F, Kirchhain A, Salvo P, Bottai D, Tavanti A, Di Francesco F: **Advances in biosensing: the CRISPR/Cas system as a new powerful tool for the detection of nucleic acids**. *J Pharmaceut Biomed Anal* 2020, **192**:113645–113665.

12. * Land KJ, Boeras DI, Chen XS, Ramsay AR, Peeling RW: **REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes**. *Nat Microbiol* 2019, **4**:46–54.

Breakthrough perspective emphasising the need for better diagnostics platform that obeys ASSURED criteria and to mitigate the failures by incorporating current technological advances, easy specimen collection and environmental compliances.

13. Xu Y, Li Z: **CRISPR-Cas systems: overview, innovations and applications in human disease research and gene therapy**. *Comput Struct Biotechnol J* 2020, **18**:2401–2415.

14. Li Y, Li S, Wang J, Liu G: **CRISPR/Cas systems towards next-generation biosensing**. *Trends Biotechnol* 2019, **37**:730–743.

15. Rashid Aman AM, Mahfouz Magdy: **Nucleic acid detection using CRISPR/Cas biosensing technologies**. *ACS Synth Biol* 2020, **9**:1226–1233.

16. Chen JS, Ma E, Harrington LB, Da Costa M, Tian X, Palefsky JM, Doudna JA: **CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity**. *Science* 2018, **360**: 436–439.

17. Wang B, Wang R, Wang D, Wu J, Li J, Wang J, Liu H, Wang Y: **Cas12aVDet: a CRISPR/Cas12a-based platform for rapid and visual nucleic acid detection**. *Anal Chem* 2019, **91**: 12156–12161.

18. Gootenberg JS, Abudayyeh OO, Kellner MJ, Joung J, Collins JJ, Zhang F: **Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6**. *Science* 2018, **360**: 439–444.

19. Sadighbayan D, Sadighbayan K, Khosroushahi AY, Hasanzadeh M: **Recent advances on the DNA-based electrochemical biosensing of cancer biomarkers: analytical approach**. *Trends Anal Chem* 2019, **119**:115609–115637.

20. Saidur MR, Aziz AA, Basirun WJ: **Recent advances in DNA-based electrochemical biosensors for heavy metal ion detection: a review**. *Biosens Bioelectron* 2017, **90**:125–139.

21. Wu Y, Arroyo-Currás N: **Advances in nucleic acid architectures for electrochemical sensing**. *Curr Opin Electrochem* 2021, **27**: 100695–100703.

22. ** Dai Y, Somoza RA, Wang L, Welter JF, Li Y, Caplan AI, Liu CC: **Exploring the trans-cleavage activity of CRISPR-cas12a (cpf1) for the development of a universal electrochemical biosensor**. *Angew Chem Int Ed* 2019, **58**:17399–17405.

Pioneering article on E-CRISPR, utilizing the trans cleavaging activity and incorporation of aptamer-based system for picomolar sensitive detection of HPV-16, PB-19 and TGF-β1.

23. Adli M: **The CRISPR tool kit for genome editing and beyond**. *Nat Commun* 2018, **9**:1–3.

24. Wang M, Zhang R, Li J: **CRISPR/cas systems redefine nucleic acid detection: principles and methods**. *Biosens Bioelectron* 2020, **165**:112430–112480.

25. Deng C, Pi X, Qian P, Chen X, Wu W, Xiang J: **High-performance ratiometric electrochemical method based on the combination of signal probe and inner reference probe in one hairpin-structured DNA**. *Anal Chem* 2017, **89**:966–973.

26. ** Bruch R, Baaske J, Chatelle C, Meirich M, Madlener S, Weber W, Dincer C, Urban GA: **CRISPR/Cas13a-powered electrochemical microfluidic biosensor for nucleic acid amplification-free miRNA diagnostics**. *Adv Mater* 2019, **31**: 1905311–1905318.

Pioneering work on microfluidic electrochemical CRIPR sensor and use of the same for clinical sample analysis.

27. ** Zamani M, Robson JM, Fan A, Bono Jr MS, Furst AL, Klapperich CM: **Electrochemical strategy for low-cost viral detection**. *ACS Cent Sci* 2021, **7**:963–972.

The authors successfully demonstrated HPV-18 DNA detection from cervical swabs sample by utilizing LAMP with CRISPR based electrochemical readout.

28. Xu W, Jin T, Dai Y, Liu CC: **Surpassing the detection limit and accuracy of the electrochemical DNA sensor through the application of CRISPR Cas systems**. *Biosens Bioelectron* 2020, **155**:112100–112106.

29. O'Connell MR, Oakes BL, Sternberg SH, East-Seletsky A, Kaplan M, Doudna JA: **Programmable RNA recognition and cleavage by CRISPR/Cas9**. *Nature* 2014, **516**:263–266.

30. Zhang D, Yan Y, Que H, Yang T, Cheng X, Ding S, Zhang X, Cheng W: **CRISPR/Cas12a-Mediated interfacial cleaving of hairpin DNA reporter for electrochemical nucleic acid sensing**. *ACS Sens* 2020, **5**:557–562.

31. Newsham E, Richards-Kortum R: **CRISPR-based electrochemical sensor permits sensitive and specific viral detection in low-resource settings**. *ACS Cent Sci* 2021, **7**:926–928.

32. ** Bao M, Chen Q, Xu Z, Jensen EC, Liu C, Waitkus JT, Yuan X, He Q, Qin P, Du K: **Challenges and opportunities for clustered regularly interspaced short palindromic repeats based molecular biosensing**. *ACS Sens* 2021, https://doi.org/10.1021/acssensors.1c00530.

Comprehensive review article on the current challenges pertaining to CRIPR based bioassays and what are the requirements need to be made it as a gold standard for biosensing.

33. * Bruch R, Johnston M, Kling A, Mattmüller T, Baaske J, Partel S, Madlener S, Weber W, Urban GA, Dincer C: **CRISPR-powered electrochemical microfluidic multiplexed biosensor for target amplification-free miRNA diagnostics**. *Biosens Bioelectron* 2021, **177**:112887–112892.

First report of multiple detection of 8 nucleic acids in a single-channel multiplexing approach.

34. Uygun ZO, Yeniay L, Sağõn FG: **CRISPR-dCas9 powered impedimetric biosensor for label-free detection of circulating tumor DNAs**. *Anal Chim Acta* 2020, **1121**:35–41.

35. Zhou T, Huang R, Huang M, Shen J, Shan Y, Xing D: **CRISPR/Cas13a powered portable electrochemiluminescence chip for ultrasensitive and specific MiRNA detection**. *Adv Sci* 2020, **13**: 1903661–1903670.

36. Li F, Ye Q, Chen M, Zhou B, Zhang J, Pang R, Xue L, Wang J, Zeng H, Wu S, Zhang Y: **Anultrasensitive CRISPR/Cas12a based electrochemical biosensor for *Listeria monocytogenes* detection**. *Biosens Bioelectron* 2021, **179**: 113073–113080.

37. Chen M, Wu D, Tu S, Yang C, Chen D, Xu Y: **CRISPR/Cas9 cleavage triggered ESDR for circulating tumor DNA detection based on a 3D graphene/AuPtPd nanoflower biosensor**. *Biosens Bioelectron* 2021, **173**:112821–112845.

38. Uygun ZO, Atay S: **Label-free highly sensitive detection of DNA approximate length and concentration by impedimetric CRISPR-dCas9 based biosensor technology**. *Bioelectrochemistry* 2021, **140**:107812–107818.

39. Sheng Y, Zhang T, Zhang S, Johnston M, Zheng X, Shan Y, Liu T, Huang Z, Qian F, Xie Z, Ai Y: **A CRISPR/Cas13a-powered catalytic electrochemical biosensor for successive and highly sensitive RNA diagnostics**. *Biosens Bioelectron* 2021, **178**: 113027–113036.

40. Cui Y, Fan S, Yuan Z, Song M, Hu J, Qian D, Zhen D, Li J, Zhu B: **Ultrasensitive electrochemical assay for microRNA-21 based on CRISPR/Cas13a-assisted catalytic hairpin assembly**. *Talanta* 2021, **224**:121878–121884.

41. Qing M, Chen SL, Sun Z, Fan Y, Luo HQ, Li NB: **Universal and programmable rolling circle AmplificationCRISPR/Cas12a-Mediated immobilization-free electrochemical biosensor**. *Anal Chem* 2021, **93**:7499–7507.

42. Liu PF, Zhao KR, Liu ZJ, Wang L, Ye SY, Liang GX: **Cas12a-based electrochemiluminescence biosensor for target amplification-free DNA detection**. *Biosens Bioelectron* 2021, **176**:112954–112960.

43. Katalani C, Booneh HA, Hajizade A, Sijercic A, Ahmadian G: **CRISPR-based diagnosis of infectious and noninfectious diseases**. *Biol Proced Online* 2020, **22**:1–4.

44. Goud KY, Reddy KK, Khorshed A, Kumar VS, Mishra RK, Oraby M, Ibrahim AH, Kim H, Gobi KV: **Electrochemical diagnostics of infectious viral diseases: trends and challenges**. *Biosens Bioelectron* 2021, **180**:113112–113131.

45. * Xiong E, Jiang L, Tian T, Hu M, Yue H, Huang M, Lin W, Jiang Y, Zhu D, Zhou X: **Simultaneous dual-gene diagnosis of SARS-CoV-2 based on CRISPR/Cas9-mediated lateral flow assay**. *Angew Chem* 2021, **133**:5367–5375.

One of the pioneering work on CRISPR/Cas in combination with multiplex reverse transcription-recombinase polymerase amplification (RT-RPA) for simultaneous dual-gene diagnosis of SARS-CoV-2 with nano gold probe.

46. ** Hosseinifard M, Naghdi T, Morales-Narváez E, Golmohammadi H: **Towards smart diagnostics in a pandemic scenario: COVID-19**. *Front Bioeng Biotechnol* 2021, **9**:510–517.

Very futuristic article on the need for using smart technology for coordinating the war, improving the rapid response, prediction and decision making against pandemic by incorporating the could computing, AI and emphasising the REASSURED criteria.

47. Kaushik AK, Dhau JS, Gohel H, Mishra YK, Kateb B, Kim NY, Goswami DY: **Electrochemical SARS-CoV-2 sensing at point-of-care and artificial intelligence for intelligent COVID-19 management**. *ACS Appl Bio Mater* 2020, **11**:7306–7325.

48. Morales-Narváez E, Dincer C: **The impact of biosensing in a pandemic outbreak: COVID-19**. *Biosens Bioelectron* 2020, **163**: 112274–112279.

49. Ibrahim AU, Al-Turjman F, Sa'id Z, Ozsoz M: **Futuristic CRISPR-based biosensing in the cloud and internet of things era: an overview**. *Multimed Tool Appl* 2020:21–36.