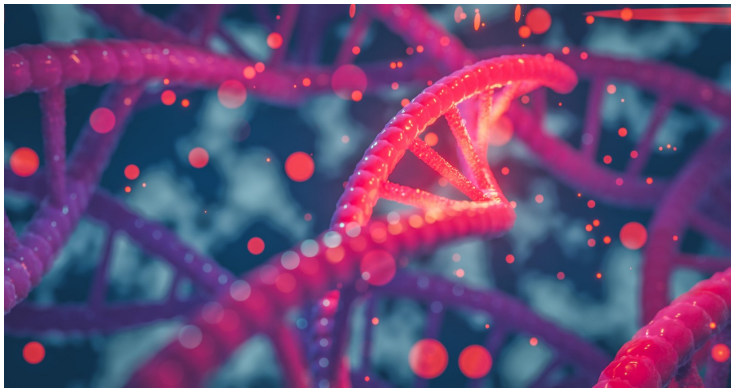
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How to Enhance the Acceptance of Versatile CRISPR Diagnostics

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CRISPR/Cas system, a popular gene editing system known for its targeted genomic cleaving capabilities, is being widely used for the development of gene edited therapies. Since 2016, CRISPR/Cas has also been extensively used for the development of molecular diagnostics and other biosensing applications. Due to targeted biosensing, CRISPR-based diagnostics can be developed as highly specific, rapid, simple, and cost-efficient tests that can be used at scale and in point-of-care (POC) settings. CRISPR-based molecular diagnostics are not only highly accurate for nucleic acid testing, but also ideal for use for POC testing as it does not require expensive and complex instrumentation or thermocycling, like PCR-based technologies. They have broad applicability, being able to detect DNA, RNA, proteins, and small molecules, making them useful in the diagnosis of infectious diseases, both viral and bacterial, and non-infectious diseases such as cancer and genetic disease via SNP and mutation detection.



While the CRISPR toolbox has been extensively researched for more than a decade, it was only in 2020 that the first CRISPR test received EUA (emergency use authorisation) from the Food and Drug Administration (FDA) for SARS-CoV-2 diagnosis. This was a landmark year for CRISPR-based molecular diagnostics, which has since then witnessed tremendous interest due to their applicability for large-scale testing for infectious diseases and beyond in resource limited settings.

DETECTR and SHERLOCK platforms, developed by Sherlock Biosciences and Mammoth Biosciences respectively, are the core platforms that were further enhanced to develop breakthrough POC SARS-CoV-2 diagnostics, which could provide results in approximately an hour. Both these platforms are versatile and can be adapted to multiple pathogens, such as Zika, Dengue, West Nile, and yellow fever virus.

CRISPR Toolbox for Diagnostics Development Beyond Infectious Disease Testing

CRISPR-based tests overcome several challenges of RT-PCR such as high costs, need for expensive reagents and sophisticated equipment, and other alternative isothermal amplification methods such as nucleic acid sequence-based amplification (NASBA), isothermal exponential amplification reaction (EXPAR), strand displacement amplification (SDA), loop-mediated isothermal amplification (LAMP) which are susceptible to false-positive results due to amplification artifacts. Adding a layer of specificity with a CRISPR/Cas system would improve the accuracy of these tests, which is key for SNP and minute levels of nucleic acid detection.

CRISPR diagnostics have been extensively developed for the detection of infectious diseases over the past decade, with application in cancer diagnosis and disease monitoring pickup as attractive applications. CRISPR diagnostic tests have also been developed for the detection of several viruses, bacteria, fungi, short microRNAs, and pathogenic proteins as an alternative to molecular diagnostics and culturing. The initial development of CRISPR-diagnostics made use of the CRISPR - Cas9 system, and the toolbox has been further improved with the discovery of effector molecules like Cas12a (targets DNA) and Cas13a (targets RNA) which expanded the applications. These enzymes have demonstrated site specific cleaving of double -stranded DNA and can cause non- specific collateral cleavage capabilities, giving them an edge over Cas9 enzymes. Apart from infectious diseases, Cas13 effectors are also being explored to detect non-infectious diseases like graft-versus-host diseases and cancer.

The highly specific and sensitive nature of CRISPR-based tests makes it a highly valuable tool for the detection of mutations and SNPs in cell-free DNA and circulating tumour cells, which are present in very small amounts in serum, necessitating a highly sensitive test. Such liquid biopsies using CRISPR would be useful to detect even minimal residual disease, and for early diagnosis using liquid biopsies.

Garnering Traction

Apart from notable names such as Sherlock Biosciences and Mammoth Biosciences which have FDA approved diagnostics for COVID-19, other companies like VedaBio, Scope Biosciences, and Crisprbits have also forayed into this space of developing robust CRISPR based diagnostic platforms. Despite its potential, CRISPR diagnostics have not taken off as expected which could be due to some of the technical and developmental challenges. Most of the R&D activity for CRISPR-based diagnostics is observed in the USA, where academia has been actively pursuing translational research. The FDA approvals for emergency use of the diagnostics also fostered a conducive environment for further development of CRISPR

tests for other applications. There is also a growing interest in this space, indicated by the funding and acquisitions observed in the last year.

Massachusetts-based Proof Diagnostics, developing POC CRISPR diagnostics was acquired by Ginkgo Bioworks in early 2024, while the Netherland-based Scope Biosciences received €2.5 million EIC Transition grant for its POC CRISPR diagnostics in 2024, exhibiting rising interest in CRISPR diagnostics in Europe. Recent research activities at the academic R&D labs in the USA that significantly boosts the use of CRISPR based applications include the development of a new search algorithm that has led to the identification of 188 kinds of new rare CRISPR systems which can be used in CRISPR diagnostics, CRISPR test for detection of monkeypox, and CRISPR test for oral bacterial pathogen detection.

Improving Adoption

Although CRISPR- based diagnostics are promising and are being increasingly researched for its utility in both infectious and chronic disease diagnosis, there are several challenges that limit the wide scale adoption of CRISPR diagnostics globally. Challenges associated with sample preparation, sensitivity, and the lack of streamlined regulatory frameworks lead the concerns.

Research community is focused on developing approaches to reduce the need for amplification and have developed preamplification free CRISPR diagnostics which are more sensitive, cost-effective and can be carried out in visual detection like colorimetric or luminescence detection methods. Strategies such as droplet based digital platforms using CRISPR for ultra-sensitive detection, cascade signal amplification and use of signal transducers have been used for amplification free CRISPR diagnostics. Optimisation of the enzymes is another area of research to improve the sensitivity of these tests.

The ease with which CRISPR can be used in rapid nucleic acid detection (POC) demonstrates a promising potential for its use in regular clinical diagnostics and at home testing. Achieving CRISPR based POC diagnostics that provides quantifiable results will be the aim of scientists in CRISPR diagnostics. In bacterial and fungal infections, where culture is still the gold standard, diagnosis, and subsequent treatment is often delayed due to the long turnaround times. CRISPR assays have shown promise in this area and can change the paradigm of tuberculosis and nontuberculous mycobacterium infections with its rapid and accurate testing protocols. It could also be a useful tool that can be used in a potential infectious disease outbreak for large scale, inexpensive testing. CRISPR diagnostics embedded with gene circuits are also being developed to make them more sensitive. miRNA detection using CRISPR diagnostics is another area of research, which would find applications in cancer testing.

The next phases of development would be around multiplexed CRISPR-diagnostics that can detect multiple targets in a single assay. Optimised assay development, along with enzyme optimisation and adoption of digital microfluidics platforms can help develop tests with high clinical utility. Its adoption could be further enhanced with the development of pre-amplification-free, POC tests. With integration into paper based and lateral flow strips, CRISPR based diagnostics can eventually be used as a simple at-home test that provides results instantly and could be even used for infection testing, pregnancy testing, urine testing and other applications.

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